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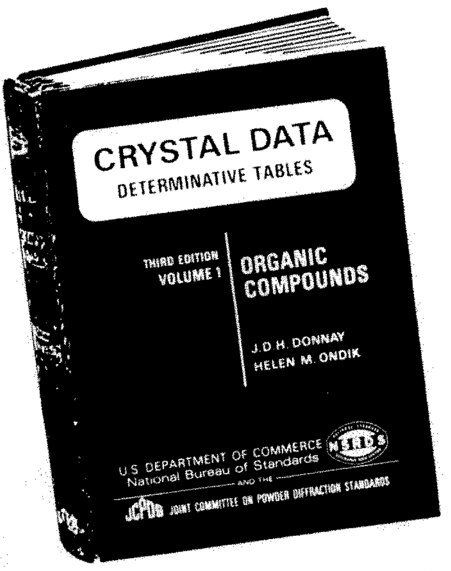
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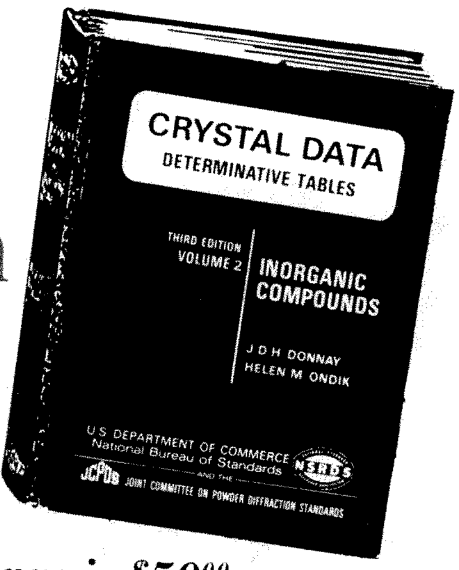
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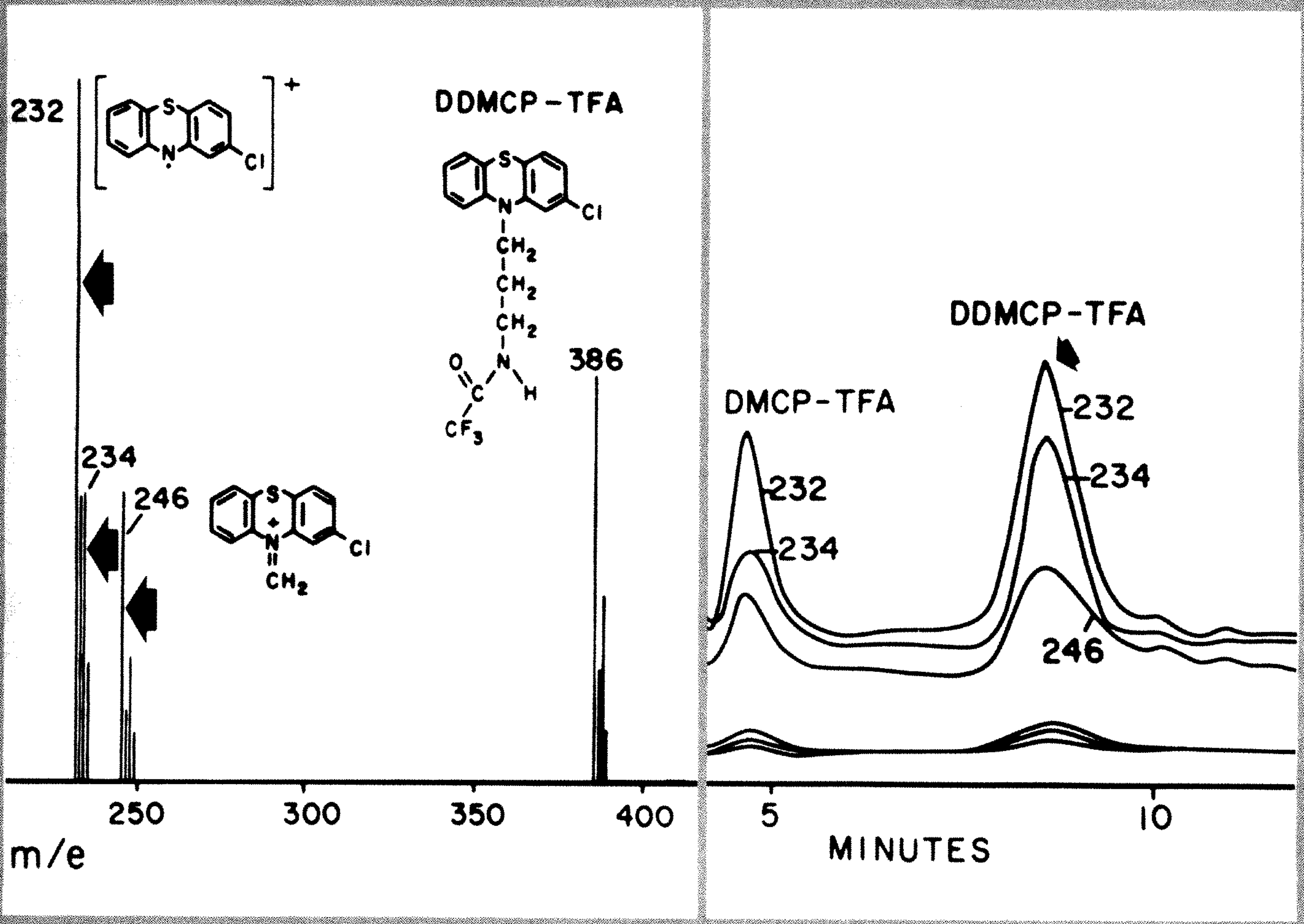

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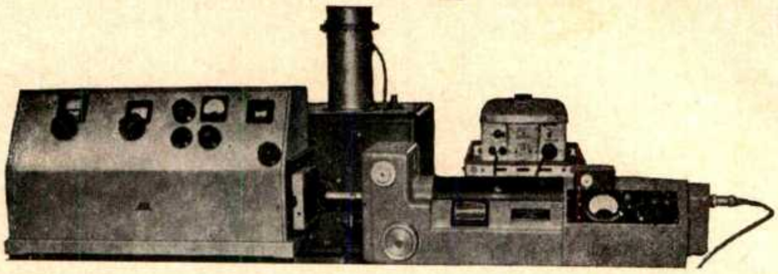

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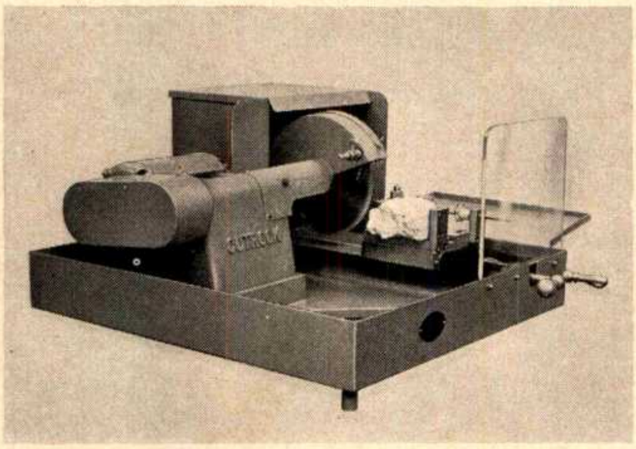

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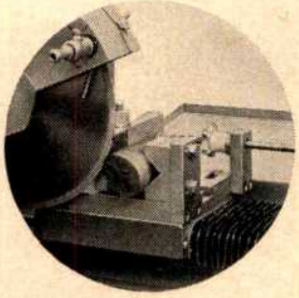

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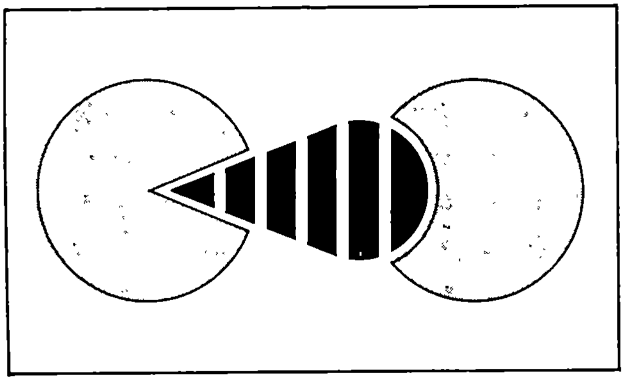

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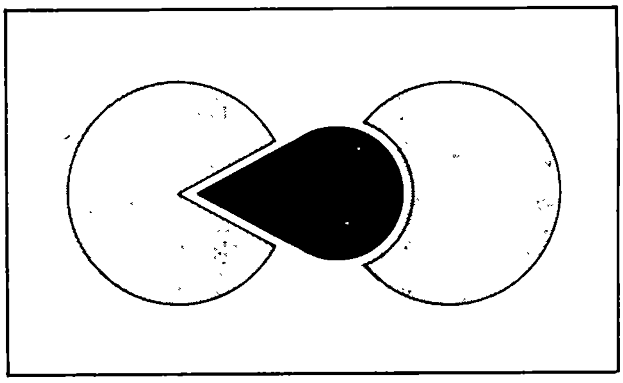

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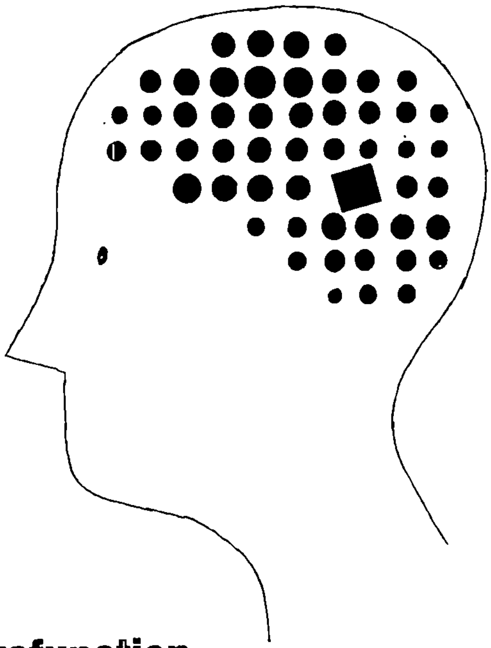

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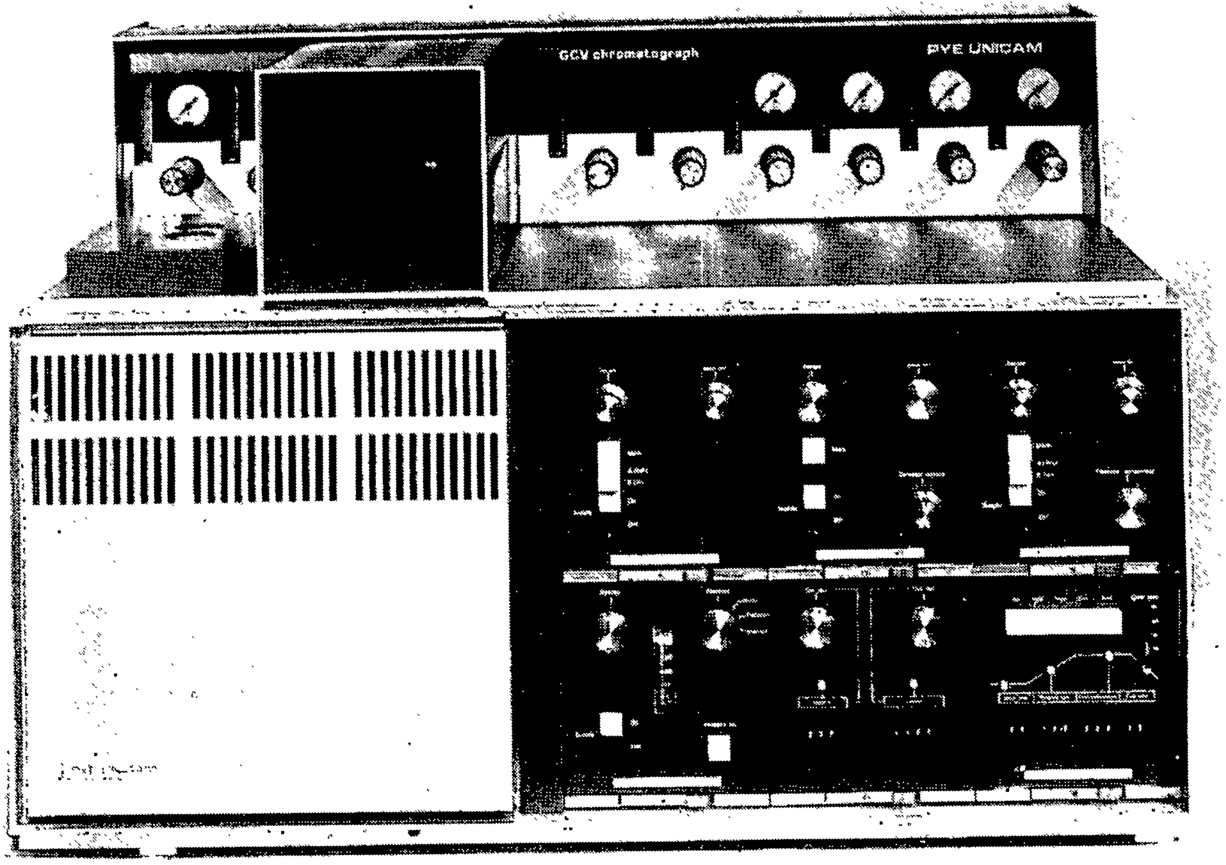

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NATURE Volume 243 MAY 4 1973

How to Fix Tranquillizer Prices

THE British government has probably stirred up more trouble than it can reasonably have expected by its decision, three weeks ago, to order Hoffmann–La Roche to reduce the prices charged to the National Health Service for the tranquillizers called (in Britain) 'Librium' and 'Valium'. For one thing, there is likely to be a court case. The Swiss company and its British subsidiary are talking of taking the issue to the House of Lords, complaining that the Department of Trade and Industry has acted illegally under the terms of the Monopolies Act which does give the British government power to regulate the prices charged to public authorities by monopoly suppliers. Nobody will dispute the truth of the findings of the subcommittee of the Monopolies Commission which, three weeks ago, argued that Hoffmann–La Roche has been making handsome profits from the sales of the two tranquillizers. The trouble is that there is no easy rule of thumb for telling when handsome profits are what the government now calls "excessive". Another difficulty is that the fact that the two tranquillizers in dispute account, on various estimates, for something like 63 per cent of the tranquillizer market in Britain, owes at least something to their cheapness compared with other comparable drugs. It may be true that Hoffmann–La Roche has promoted its products vigorously, but it is also true that two-thirds of the patent protection it has enjoyed has now expired. Whether the case goes to the House of Lords, and whatever may be the outcome, there is at least a strong case for thinking that the government would have been wiser to respond to the report of the Monopolies Commission by taking action under the patent legislation than by dubiously enforcing one of the provisions of the Monopolies Act. At the very least, it is unseemly that a monopoly customer should use its own legislation to enforce a chosen pattern of behaviour on a somewhat less monopolistic supplier.

This said, it is very much to be hoped that the Hoffmann–La Roche case will not be allowed to sink into oblivion. To begin with, it brings sharply to the surface the question of how governments such as the British, now a member of the European Community, are to deal with what used to be called multinational companies but which are now European in some strict sense (for Switzerland is not, after all, that far from Brussels). In many ways, the issue of how Hoffmann–La Roche should conduct itself is a better precedent for the good conduct of European multinational companies than the recent decision of the European court and the expansionist proclivities of the Continental Can Company, an essentially American organization which has nevertheless been allowed to establish strong manufacturing organizations in Europe because there is no basis in equity for saying otherwise. In other words, it is to be hoped that if Hoffmann–La Roche gets no joy in the House of Lords, it will have a try in the European court. What governments like the British have to decide is where they stand on the relationship between governments and private enterprise.

Pharmaceutical manufacturing is by any reckoning a speculative business, not much different from the business of drilling holes in the ground in the hope of finding gold. Pharmaceutical manufacturers are inclined to imply that their speculative expenditure is sanctified by the name research, and that it is therefore more virtuous than what people in Texas call "wildcatting". But there is not very much difference, and indeed the willingness of pharmaceutical companies to invest large sums of money in the development of new drugs is likely to persist for so long as those who need or use drugs consider it to be inestimably valuable to have something that performs better, even a tiny bit better, than what exists at present. In circumstances like these, the British government, which has fired the first shot at Hoffmann–La Roche, ought at the very least to be asking itself whether it should not now take action against all those entrepreneurs who have in the past few years bought tiny plots of land and found them appreciating enormously in value, who have invented new devices for helping to make computer systems work more efficiently and then found themselves rich beyond their expectations. For what the government has done over 'Librium' and 'Valium' is to say that even in a strictly speculative field of enterprise, profits must never be immoderate.

It would be much more sensible if the British government were to take a simpler and more radical view of the problem which confronts it. For practical purposes, the National Health Service is a monopoly customer for drugs, and the British government is well placed to act as a customer on the international market, seeking out the drugs which the National Health Service needs, making arrangements with the patent holders to manufacture under licence, and then arranging to manufacture. There is no valid reason why the National Health Service should not be somehow closely involved with the machinery for providing itself with essential supplies except that successive governments have always fought shy of anything that smacks of interference either with the medical profession, pharmaceutical manufacturing or the high street chemists. But the truth is that the National Health Service and the British taxpayer might have had a much better deal if the government had recognized its responsibility in this field and had taken a firm grip on the machinery by which drugs are at present supplied. What this implies is that the arbitrary action against Hoffmann–La Roche, populist though it may be, is not even the first step towards the solution of the problem which the government must sooner or later tackle.

Burn More Coal

THE British government has quickly echoed the United States government with a modest response to the increasing scarcity and price of petroleum. First of all, it has been agreed that the Central Electricity Generating Board will in the coming year burn more coal in British power stations than would be dictated by simple considerations of price. To make sure that British power stations do not turn to petroleum, at present prices a somewhat cheaper fuel than coal, the government has agreed to pay a subsidy of £1.75 a ton for the extra coal burned at power stations in the coming year. The decision is meant to cover only a limited period of time, for plainly there is at least a chance that the growing American demand for Middle East petroleum and the increasing toughness of the oil producing countries will bring about further increases in the price of petroleum in the coming year, so that a much smaller subsidy, or perhaps even none at all, will be appropriate in 1974. Whatever the future may hold, the decision is, however, a great convenience for the coal industry itself—without a subsidy for power station coal, there would have had to be a further contraction of coal mining in Britain in the months ahead, and the British government is well aware that each million tons of coal represents the jobs of 2,000 people under British conditions. In other words, the coal subsidy is as much a part of the British government's new attitude towards industries and groups of workers in trouble as of a deliberate energy policy as such.

It is all very well for the British government to live from hand to mouth in ways like these—indeed there is some virtue in a pragmatic approach to the problem of how to ensure adequate supplies of fuel in the years ahead. At the very least, the government needs some way of anticipating the consequences of sharp increases in the price of petroleum in the months ahead. It would, of course, be a great waste of resources to be shutting down coal mining operations this year and then paying prices for petroleum next year in excess of what coal would have cost. In other words, if the subsidy for the extra coal now to be burned in power stations is simply a bridging operation, there is no objection to it. The danger is that it will become sanctified with the passage of time and converted into a general undertaking that the British government will support the British coal industry at the level to which the National Coal Board and the country's coal miners are now accustomed. And the truth, of course, is that in spite of all the considerable improvements of technical efficiency, and the increases of productivity which have resulted, British coal is still comparatively expensive. Certainly it is an exceedingly costly commodity alongside, say, coal from the productive coal seams of the United States. Moreover, coal mining remains a relatively labour intensive industry, which implies that the real cost of coal is bound to increase more quickly than the prices of other commodities as real incomes increase. This is why the British government should not be alarmed by the fear of increased American competition for Middle East oil and rapacity on the part of OPEC countries into a policy involving a minimization of purchases of petroleum from the Middle East. After all, in the next few years it is entirely possible that the position of the OPEC countries will be turned by the discovery of substantial deposits of petroleum elsewhere than in the Middle East.

At the same time, it is fair to say that the British government, like the United States Administration a week ago, has said too little about its plans for the support of research and development in other fields of energy supply. President Nixon, to be sure, did say in his energy message to Congress that expenditure on thermonuclear fusion would be increased, and this is entirely appropriate. It is welcome that the corresponding British programme is likely also to be more fully supported in the months ahead. But is there not a case for a much more closely coordinated and sharply pointed programme of research and development on breeder reactors, which are bound to have to be, in the 1980s and the 1990s, the work horses of nuclear energy? And is it not high time that some of the more futuristic schemes for extracting energy from, for example, the surface layers of the oceans which are heated by solar radiation, were investigated properly?

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100 Years Ago

A VOICE FROM CAMBRIDGE

IT is known to all the world that science is all but dead in England. By science, of course, we mean that searching after new knowledge which is its own reward, a thing about as different as a thing can be from that other kind of science, which is now not only fashionable, but splendidly lucrative—that "science" which Mr. Gladstone and Mr. Lowe always appeal to with so much pride at the annual dinner of the Civil Engineers—and that other "science" prepared for Jury consumption and the like.

It is also known that science is perhaps deadest of all at our Universities. Let any one compare Cambridge, for instance, with any German university; nay, with even some provincial offshoots of the University in France. In the one case he will find a wealth of things that are not scientific, and not a laboratory to work in; in the other he will find science taking its proper place in the university teaching, and, in three cases out of four, men working in various properly appointed laboratories, which men are known by their works all over the world.

This, then, is the present position of Cambridge after a long self-administration of the enormous funds which have been so long accumulating there for the advancement of learning. Cambridge no longer holds the place which is hers by right in the van of English science, her workers are few, and to those few she is careful to afford no opportunity of work, such as it is the pride of scholastic bodies in other countries to provide for the men who bring the only lasting honour to a university.

From Nature, **8**, 21, *May* 8, 1873.

OLD WORLD

ELDO Ends Europa II Programme

THE European Launcher Development Organization (ELDO) has abandoned its work on the Europa II launcher. The ELDO council was left with no alternative but to terminate the project following an announcement by France and Germany that they would not be able to support it from May 1. As most of the finance comes from these two countries the council directed the Secretary General of the organization to close down the projects and inform those companies which hold contracts from ELDO that the programme has ended.

The decision leaves the 9-year-old organization with no current programme and no immediate prospect of one, although its legal framework still exists.

The closure of the programme follows the cancellation of the Europa III project last year and the failure of F11, the first test firing of Europa II in November 1971. In all, ELDO has launched eleven rockets, only six of which have been completely successful, chiefly in the early stages of the development of Europa I.

Precisely what will happen to the organization is not yet clear. ELDO employs a few more than 300 people in Paris and at Kourou in French Guiana, ELDO's test centre, some of whom are certain to be made redundant. The member countries of ELDO will have to decide what is to happen to the organization, and the likeliest possibility at present is that it will be restructured with the intention of retaining those personnel with expertise in launcher development, and organizing the liquidation of the Europa project. But quite what, if anything, the organization will do between now and January 1, 1974, when it is to be merged with the European Space Research Organization in a European Space Agency is not clear (see *Nature*, **240**, 516 ; 1972).

The decision to end the Europa programme leaves Europe with no plans to develop a launcher other than those of the French to build the L3S rocket. This was proposed as the alternative to Europa III and approved in principle by the European Space Conference last December, although no funds other than from France have yet been put into it. It is possible, if the French can persuade the Germans to participate, that the new agency will develop LS3 as a cooperative venture, using some of the expertise assembled within ELDO.

But there are even doubts about the continued viability of L3S for, although there is some provision for developing the launcher in the French budget this year, enthusiasm for the project may have waned somewhat now that M. Michel Debré, the launcher's chief protagonist, is no longer Minister of Defence following the French general elections.

The decision to end the Europa project typifies the continuing disagreement within Europe on the subject of launchers. Germany (and Britain) have long advocated buying launchers from the United States as and when they are needed. The French, on the other hand, argue that Europe must maintain an independent capability, and it is this that led to the proposal to develop LS3, a launcher with similar capabilities to Europa III but which, the French claim, could be built at about two-thirds of the cost.

But whether L3S is developed or not, the French, the Germans, and the European Space Research Organization are all going to have to rely on the United States to launch Symphonie, the Franco-German communications satellite, and COS-B, ESRO's next scientific satellite, both of which were due to be launched on Europa II.

DRUGS

Safety or Service?

CONCERN over the safety of new drugs must not be allowed to get out of proportion, according to Mr Ivar Boden, retiring president of the Association of the British Pharmaceutical Industry. Mr Boden, speaking at the annual dinner of the association recently, said that it is unlikely that entry into the European Economic Community will markedly change the strategy of the chief British drug companies. He hoped that Britain would not be forced to change its already well established system for

---

ROYAL INSTITUTION

Healthier Situation

IN spite of grave predictions made a year ago the Royal Institution showed a slight profit for its operations during 1972. When the 1971 annual report came out showing a deficit of £7,812, the then newly appointed treasurer, Sir Gordon Cox, forecast a deficit of £17,600 in 1972. But economies in nearly all the Royal Institution's operations combined with a few benefactions resulted in a profit of £5,486 last year.

The figures for 1971 and 1972, however, are not directly comparable because a new system of classifying the accounts and a new system of treating the money available for research have been introduced. The net result is that the Royal Institution is more than £16,000 better off this year than last.

The process of selling off books which were not considered essential has now been concluded and £150,000 has been raised which has been invested for the benefit of the library. The interest from this money was £8,575 in 1972 and more will be expected this year from this source.

The membership drive, which was expected to increase the membership by 300 a year, failed to reach this target because 50 members either died or did not renew their subscriptions—the net increase was 250.

One of Sir Gordon's aims is to make the membership activities self sustaining—last year there was a deficit of £3,268 in this sector. But the trend is most satisfactory and it is hoped that these activities should, at least, break even during 1973.

Apart from economies in most activities, one of the other reasons for the accounts looking much more healthy in 1973 is an anonymous gift of $188,000 over ten years to be used to provide administrative assistance and services for the director, Sir George Porter. In 1972 this fund added £5,846 to the accounts. Also during 1972, Applied Photophysics Limited contributed £4,027 to the Royal Institution.

But what of the future? During the past ten years the Royal Institution has accumulated a deficit of about £50,000. Sir Gordon Cox said this week that it is his intention to make good that sum in the next ten years. But because of inflation, twice this amount will have to be recouped before the finances are in the same situation as they were in 1963.

As far as 1973 is concerned several effects make predicting the financial position at the end of the year uncertain. First, the effect of corporation tax is unknown, and Value Added Tax will take about £2,000 of the Royal Institution's funds. Because of the changed system of income tax in Britain the institution will probably suffer a decrease in income, estimated to be about £500, from recovered income tax covenants.

licensing medicines, and he emphasized that the industry must still regard the possible dangers of new drugs with the utmost concern.

But while products should continue to be manufactured with safety very much in mind, Mr Boden said, safety must not become too much of a threat to the progress of science. If this happens it will take much longer to pass on the benefits of research to society.

Those who are demanding that new drugs be absolutely safe before release to the public are pursuing a myth, according to Mr Boden. The cry of safety at all costs is impractical and the cost of research must not be raised to a level that no manufacturer can afford.

There are signs that pressure is being brought to bear to slow down the work of the Committee on Safety of Medicines, and it would be sad, said Mr Boden, if the British pharmaceutical companies had to look outside this committee for somewhere to carry out early clinical trials on drugs.

People must, of course, have a right to obtain adequate compensation for injury sustained from drugs, Mr Boden said, but the sum of nearly £1 million a day that is spent world wide in research by the pharmaceutical industry must be used to optimum advantage.

SPACE

Copernicus-500

The joint Polish–Soviet solar physics research satellite, promised last February as part of the Copernicus quincentenary celebration, was launched on April 19 as part of the Interkosmos series. The satellite is designed to study "sporadic" solar radiation, in particular the radio emission from the Sun between 0.6 and 6.0 MHz and also to investigate the characteristics of the ionosphere. Its orbital parameters are: perigee 202 km, apogee 1,551 km, inclination 102.2°.

"Copernicus-500", as this special Interkosmos is called, carries low-frequency and high-frequency ionosphere probes of Soviet manufacture, and a radiospectrograph developed by a Polish team based in Toruń, the birthplace of Copernicus. This is the first time that this Polish instrument has been used in the Interkosmos programme, although similar radiospectrographs have been carried by the Comecon high-altitude geophysical probes.

Interviewed in *Pravda*, the head of the Polish side of the Copernicus project, Academician Dionizy Smoleński, spoke of the particular contributions which Poland hopes to make to the Comecon space projects. Four chief fields are involved: space physics (including space geodesy), meteorology, communications and space biology and medicine. In particular, Polish scientists are playing a considerable role in the *Dinamika* programme which comprises a number of experiments on the Earth's gravitational field, undertaking the coordination of theoretical and experimental work on the improvement of methods of observing and computing the trajectories of space objects for geodesic purposes.

Though "Copernicus-500" was announced as a joint Polish–Soviet experiment, the other Comecon countries are also participating. Czechoslovakia has provided a special on-board telemetry transmitter, and synchronized solar observations are being made from astrophysical and geophysical observatories throughout the Comecon block.

EDUCATION

Social Significance

from a Correspondent

An association to further the social significance of the teaching of science and mathematics in the British Commonwealth has been set up as a result of a meeting of representatives of seventeen countries which was held at the University of the West Indies at Kingston, Jamaica, last month.

The acting chairman of the association (Commonwealth Association of Science and Mathematics Educators, CASME) is Mr Maurice Goldsmith, chairman of the Guinness awards, which organized the symposium.

One of the aims of the association is to ensure that the natural sciences, technology, mathematics and the social sciences are integrated in the training of teachers. This, however, requires that teachers must be trained in a different way. CASME will consider, among other topics, how this can best be done.

LUBRICATION

Contract for Swansea

The Tribology Centre at the University College of Swansea has been awarded a contract by the European Space Research and Technology Centre to look into the use of molybdenum disulphide as a lubricant. Although the compound has been used in the United States' space programme, for example to lubricate the legs of the Apollo lunar modules, European designers have been reluctant to use it because there is a conflict in the literature about its suitability in space conditions.

Dr A. R. Lansdown, Director of the Tribology Centre said this week that although the contract is a preliminary one worth less than £10,000 he is hopeful that it will be followed by others.

NATURE CONSERVANCY

New Council Chairman

Sir David Serpell, former Permanent Secretary at the Department of the Environment, is to be the first chairman of the new Nature Conservancy Council which is due to come into existence shortly.

The new council, first outlined in last July's white paper on Government research and development, will take over part of the functions of the Nature Conservancy, and will be responsible for establishing, maintaining and managing nature reserves in Britain, and commissioning and supporting relevant research. The council will have a budget of about £1.4 million, £0.3 million of which will be spent on research. The remainder of the Nature Conservancy's work will remain within the Natural Environment Research Council, of which the conservancy is a part, and a new Institute of Terrestrial Ecology will be formed by the council.

Sir David Serpell

Sir David, 61, who retired in September last year, has been a civil servant for more than thirty years, working at various times for the Ministries of Food, Fuel and Power, and Transport, with spells at the Treasury and the Board of Trade. In 1970 when the Department of the Environment was formed, he became its first permanent secretary. A graduate of the University of Oxford, the University of Toulouse and Syracuse University in the United States, he is also a fellow of the Fletcher School of Law and Diplomacy in the USA.

NEW WORLD

Public Accountability and the Academy of Sciences

by our Washington Correspondent

THE National Academy of Sciences, the most prestigious scientific organization in the United States, was sharply criticized last week for allowing some of the committees which advise the government in its name to be "subverted by special interests". The charges were made in a speech delivered at the annual meeting of the American Physical Society by Philip M. Boffey, who for the past two years has been conducting an investigation of the academy for the consumer advocate Ralph Nader. Academy officials have responded to Boffey's charges by suggesting that they are aware of the problems to which he referred and that steps have already been taken to overcome them.

Unlike the Royal Society of London, whose chief purpose in life is to elect new members, the National Academy of Sciences is perhaps the most important source of supposedly independent scientific advice to the federal government and thus charges of bias are not to be taken lightly. The process by which the academy advises the government works like this: Federal agencies which need help with a problem contract with the academy for advice, a group of experts (not necessarily academy members) is appointed through the National Research Council —the Academy's operating arm—and ultimately a report is produced for the contracting agency. At a recent count, some 8,300 scientists were advising the government through academy committees on problems ranging from the SST to the use of chicken feathers to stuff army mattresses.

Boffey told the American Physical Society that two chief themes have emerged from his study, namely that some academy committees "fall captive to the thinking of the government agencies which contract for their services", while others sometimes "fall prey to industrial interests". He illustrated these charges with examples of the workings of two academy committees, drawn from several case studies which will be documented in his final report to be published later this year.

Boffey suggested in his speech, and also in an interview afterwards, a number of situations in which the academy can unwittingly become aligned with the agency which contracts for its services. First, there is the old Washington trick of appointing a prestigious study group as a means of heading off public criticism of a programme. Second, he suggests that academy committees are sometimes inclined to pull their punches so that they do not lose future contracts with that particular agency. And finally, he maintained that the practice of showing draft reports to the agency before final publication "has a subtle eroding effect on the committee's ability to be critical".

As illustration, he pointed in his speech to a committee set up in 1964 to study the possible environmental problems associated with supersonic transporters. The committee was set up under contract with the Federal Aviation Agency in the aftermath of public opposition to sonic boom tests in Oklahoma, and the committee's first report "incredibly . . . recommended that the government conduct a public relations campaign to persuade people to accept the SST". The conclusion of a 1968 report from the committee that "the probability of material damage being caused by sonic booms generated by aircraft operating in a safe, normal manner is very small" eventually led 189 academy members to sign petitions contending that the report was inaccurate and should be corrected. No such correction was made public, however, and similarly a note produced by the committee "clarifying" its report and qualifying its chief conclusion was also never publicly issued.

As for bias towards industry, Boffey chose as an example the Food Protection Committee, which has already had a rough time at the hands of critics in testimony to Congress and in newspaper articles. The committee and its subpanels, Boffey said, "reflect a consistent bias—they are lenient about recommending action that might restrict the use of chemical additives in the

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NATIONAL ACADEMIES

Divorce Imminent

by our Washington Correspondent

FOR the past nine years, the National Academy of Sciences and the National Academy of Engineering have been on the verge of divorce, but the split now seems about to be made final. The membership of the National Academy of Engineering is being asked by the academy's governing council to vote this week on a resolution that the NAE be incorporated independently of the NAS and that a foundation be set up to raise money to enable the NAE to carry on its work of advising the government on questions of engineering and public policy. The membership must agree to the resolution at meetings held on May 3 and October 24, before the divorce becomes final.

The split ends nine years of fruitless efforts by the councils of the two academies to work out a mechanism to allow the academies to share in the governance of the National Research Council, and the divorce is being greeted with mixed feelings. Set up in 1964, the NAE has been operating under the charter of the NAS, and the original intention was that the two academies would work together in giving advice to the executive branch of the federal government. But the NAE has set up its own network of advisory committees instead of working through the National Research Council, partly because the control of the research council is vested solely in the hands of the council of the NAS.

A part of the problem has been mutual distrust between the two academies, with NAS members being distrustful of conflicts of interest that arise when the corporate executives who are members of the NAE serve on advisory committees, and members of the NAE being unwilling to work in an organization whose management is out of their hands. (And the distrust felt by members of the NAS has been sharpened recently by the election of Robert Seamans Jr., secretary of the Air Force, as President of the NAE. Seamans, who will step down from his government post before assuming the presidency, is regarded by his critics as an integral part of the military–industrial complex.)

Although there will be members of both academies who welcome the divorce, it is a keen blow to attempts to keep the NAS a multidisciplinary body able to offer advice on broad social questions. The NAS annual meeting passed a resolution last week, however, extending an invitation to the NAE "to work together to develop effective means for the two academies to cooperate in the national interest on activities of mutual and overlapping concern". Clarence H. Linder, the present president of the NAE has already stated his hope that the two academies will be able to work together.

food supply and they downplay the likelihood of such long-term hazards as cancer, genetic damage and birth defects".

He suggests two chief reasons for the food protection committee's alleged bias. First, it has close ties to industry through its funding—in 1972, 40 per cent of the committee's budget was provided by sources in industry—and through an industry liaison panel which gives industry "a privileged voice in committee affairs". And second, the committee's panels have seldom contained experts in mutagenesis, teratogenesis or carcinogenesis.

Asked last week for his comments on Boffey's charges, Dr Philip Handler, President of the National Academy of Sciences, called his speech "the greatest accolade we have received yet", the rationale behind that remarkable conclusion being that the two examples chosen by Boffey are well known and that the academy has already taken steps to deal with such problems. Handler argued that the academy has about 500 committees and if Boffey can only dig up those two "old chestnuts", there cannot be too many skeletons in the cupboard. Boffey points out, however, that those two examples were drawn from many which will appear in the final report.

Handler also drew attention to several reforms which the academy has recently instituted in an attempt to eliminate bias from its committees and to prevent the committees becoming too cosy with the contracting agencies. From January this year, for example, every committee member must sign a statement giving details of employment and consultantships in the past 10 years, research support during the past five, current financial interests and any publicly stated positions on the subject matter before the committee. To help overcome the problem of committees becoming aligned with the contracting agency, Handler pointed out that the academy has instituted a rule that members are appointed for fixed terms of three or four years and that reappointments must be closely justified.

But perhaps the chief reform instituted in recent years has been the setting up of a Report Review Committee under the chairmanship of Dr George Kistiakowski. The committee examines each report issued in the academy's name for scientific accuracy, possible bias and ambiguous phrasing, and suggests changes both to the committee concerned and to Handler. The committee consists of academy members.

But Boffey suggested that although such reforms are "worthwhile palliatives to the basic problems, they are by no means the complete answer", and likened the process to that of car manufacturers cleaning up exhaust emissions by fitting add-on catalysts instead of developing a non-polluting engine. As for bias statements, Boffey pointed out that many members of panels of the Food Protection Committee have come under attack for potential sources of bias but they are still serving.

The provision that members of committees should serve for fixed terms is a "totally inadequate response" to the problems of committees becoming too cosy with the contracting agency, Boffey suggested. It does not, for example, attack the problem of staff members of the academy being anxious to preserve contracts with the agencies, and Boffey said that he has "evidence of subservience to the executive branch as of two days ago".

As for the report review committee, Boffey says that "is one of the best things that has happened to the academy in recent years. It is a big step in the right direction". But even so, he believes that the committee could be made much more effective if its reports and comments were made public. He cited in his speech an example of a report by the Food Protection Committee on *Guidelines for estimating toxicologically insignificant levels of Chemicals in Foods* which was savagely attacked by a group of cancer experts, whose views were acceptable to the review committee. But the review committee's comments have never seen the light of day, and the academy is still in the position of seeming to support recommendations with which one of its most distinguished committees disagrees.

Essentially what Boffey is asking for is more public accountability in the academy. His study, the first independent investigation of the academy since it was founded during Lincoln's presidency in 1863, may help push the academy in that direction.

AMERICAN PHYSICAL SOCIETY

Pyrrhic Defeat

by our Washington Correspondent

The membership of the American Physical Society has rejected a proposed amendment to the society's constitution which would have added to the present aims of the society, which are simply "the advancement and diffusion of the knowledge of physics", the words "in order to contribute to the enhancement of the quality of life for all people. The society shall assist its members in the pursuit of these humane goals and it shall shun those activities which are judged to contribute harmfully to the quality of mankind". Proposed by Robert H. March, a nuclear physicist from the University of Wisconsin, the amendment was defeated by 45,000 votes to 37,000.

March said last week that he is neither surprised nor disappointed at the vote, because the resolution has essentially become redundant. The society is already engaged in discussions of social responsibility through its newly-established Forum on Physics and Society, he said, and the council has come a long way during the past year towards agreeing with the views of several supporters of the amendment who simply wanted such issues debated. March himself was unhappy about the wording of the last part of the amendment, which has borne the chief brunt of the criticism, and at one stage was prepared to drop the part instructing the society to shun some kinds of activities (see *Nature*, **240**, 518 ; 1972). Legal difficulties prevented alteration of the proposed amendment, however.

Short Notes

Classified Research

Dr Edward Teller, so-called father of the US H-bomb, last week urged Congress to pass a law requiring all secret government scientific research to be declassified a year after it is completed. Speaking at the annual meeting of the American Physical Society, Teller argued that secrecy hinders the progress of science and urged government officials to pay more regard to the damage resulting from overclassification than to the damage likely to result from making research results public. He also suggested that the efficiency of intelligence operations makes classification pointless for longer than a year, and included weapons design in his definition of scientific research.

DES Banned Again

When the Food and Drug Administration banned the use of the synthetic hormone diethylstilboestrol (DES) in animal feeds last year, it did not prevent farmers from implanting pellets of the hormone in the ears of cattle. Used as a growth promoter, DES produces the same effect when used as an ear implant as when added to cattle feeds. But last week, the FDA banned that use as well, making the ban on DES as an agricultural chemical complete. The hormone was taken off the market as a feed additive last year because radioactive tracer techniques showed that it remained in beef liver for up to 120 days, and thus could not be prevented from turning up in the meat supply. Since the hormone is highly carcinogenic, the FDA had no alternative but to ban it. The same sensitive tracer studies have now been applied to ear implants, and not surprisingly they have turned up the same results, hence the ban last week.

NEWS AND VIEWS

Going in the Red

RHODOPSIN, the visual pigment of retinal rods, is a molecule that has caused many a theoretician to shed tears of bitter frustration. Its chemistry, thanks largely to the work of Wald and his colleagues, is understood in its essential details, but its absorption spectrum is all but unexplained. When the carotenoid prosthetic group, 11-*cis* retinal, which is pale yellow, is bound to the protein, opsin, an enormous spectral shift ensues, with the appearance of a red, or in the case of the cone pigment, iodopsin, a purple colour. To account for such a formidable wavelength shift—200 nm or even more—has taxed even the fertile imagination of the spectroscopists. There is good evidence that the terminal aldehyde group of the retinal is bound by way of a Schiff base linkage to the amino group of a lysine side chain in the protein, and an increase in the length of the π-electron system, with a consequent redshift in the absorption band, would result if a protonated aldimine group were added to the end of the carotenoid system.

Now the electronic spectra of the carotenoids are as well understood as anything in molecular spectroscopy, and certain it is that the extension of the π-system by this much can explain only a small part of the observed shift, as indeed is also experimentally obvious from the spectra of model compounds. Explanations of the spectra of the visual pigments, which directly determine the wavelength-dependence of the visual response, have therefore tended to invoke perturbations, unknown to science in any other context, involving special dispositions of charged groups around the chromophore, massive induced dipoles, and so forth. After all these years of whistling in the dark, an altogether more convincing hypothesis has at last appeared. This is propounded by Mendelsohn, writing in this issue of *Nature* (page 22), and with hindsight, it seems perhaps slightly surprising that this perception has not come sooner.

Mendelsohn has not actually worked with a retina pigment, but rather with the closely related material from the purple membrane of the halophilic bacterium, *Halobacterium halobium*. The chromophore, as in rhodopsin and iodopsin, is 11-*cis* retinal; it has been shown to be attached to the protein through a Schiff base with a lysine amino group, and its absorption spectrum is in essence that of a visual pigment, for which it may therefore be regarded as an analogue. The advantage of this material is that it is much more stable to light, and is not in fact photochemically bleached. This makes it a promising object for study by the resonance Raman technique, which, it has been lately pointed out, offers certain unique advantages in regard to chromophoric biological systems. The principle is that when the exciting line falls in the electronic absorption band, and there are no complications from fluorescence or photochemical lability, Raman-active frequencies of the chromophore are greatly enhanced in intensity, and the spectrum can therefore be examined at very low concentration, and without interference from the contributions of other species, the intensities of which are by comparison infinitesimal.

Thus laser excitation within the absorption band of the purple membrane leads at very low total pigment concentration to a Raman spectrum containing some fifteen peaks, which vanish if the pigment is first bleached by denaturation with cationic detergent. The most prominent band lies at 1,531 cm^{-1} and its relative intensity increases as the excitation wavelength approaches the visible absorption maximum. A second intense band at 1,568 cm^{-1}, on the other hand, becomes more intense when the excitation wavelength shifts towards the blue. It is inferred that the two vibrations are coupled respectively to the longest-wavelength electronic absorption band, and to a transition at shorter wavelength. All other vibrations behave like the 1,531 cm^{-1} band. With the aid of published assignments for the Raman spectra of retinal derivatives, the principal features in the resonance Raman spectrum can be identified, in particular those in the region containing C=C and C=N stretching modes. Comparison of the observed frequencies and their dependence on deuteration, with those of model compounds, makes it clear in the first instance that the chromophore contains an unprotonated Schiff base, which at once annihilates a widely held hypothesis. The most striking observation, however, is that the dominant 1,531 cm^{-1} band has no counterpart in the model compounds, and is surmised therefore to arise from perturbation of the π-electron system with some other element of the protein. Moreover the system is highly sensitive to solvent: addition of chloroform induces a reversible shift of 60 nm in the electronic absorption band, with a corresponding displacement of the intense Raman line.

All this is diagnostic of a charge-transfer interaction, and the best candidate for an electron donor in the protein is, as Szent-Györgyi perceived many years ago, the indole chromophore of tryptophan. Retinal and tryptophan when mixed in solution do indeed give rise to a new long-wavelength absorption band, indicative of the formation of a charge-transfer complex. The conclusion that the absorption band of the visual pigment arises in this way has yet, of course, to be proved, but its attractions and the several hitherto perplexing features of the system for which it at once provides an explanation render it a compellingly attractive proposition. It may moreover give pleasure to those spectroscopists who rallied fifteen years ago to Szent-Györgyi's banner, and set off in pursuit of Submolecular Biology, for it is not often that a charge-transfer complex turns up in nature, without a sprinkling of chloranil or iodine from the hand of the Master.

From our Molecular Biology Correspondent

Paradoxical Effect of ALS

RECENT advances in cellular immunology seem to have provided an understanding of the array of cells involved in immunological responses. It seems that many immune responses involve the participation of at least three kinds of cell—T lymphocytyes of thymic origin, B lymphocytes of bone-marrow or bursal equivalent origin and macrophages. In some situations it is possible to deduce that the activities of T cells lead to the production of humoral factors which, when bound to the macrophages, can stimulate B cells to produce antibody. The net results thus seem to be attributable to the activities of an immunological orchestra. It may also prove to be the case that the responses to some kinds of antigenic stimulus involve only small sections of the orchestra. Clearly it will often be a complicated matter to gain a concise interpretation of the effects of an extrinsic immunosuppressant on such an intrinsically complex function. A report by Cantor and Asofsky on page 39 of this issue exemplifies this.

Antilymphocyte antiserum (ALS) has been found to be a potent immunosuppressive agent in a variety of animals. It is often supposed that its effect is caused by selective depletion of T cells and that it has little influence on the thymus as a source organ. There are, however, reports which have indicated an effect on bone marrow, on the thymus and on macrophages and thus any simplistic view of the mode of action of ALS is probably wrong. The variety of results can in part be attributed to the variety of methods used to produce ALS and the variety of the producing animals. Cantor and Asofsky dealt with ALS produced according to a recipe of Levey and Medawar (*Proc. US Nat. Acad. Sci.*, **56**, 1130 ; 1966). They injected this material, in relatively small amounts, into parental mice. At various times later the capacity of the thymuses of these animals to engender a graft-versus-host (GVH) reaction in newborn F_1 recipients was determined.

Cantor and Asofsky found that the GVH activity of the thymocytes of recipients of ALS was much increased for about the same time that the comparable activity of the spleens of the same animals was reduced. There was no evidence of a reduction in the cellularity of the thymuses of the ALS-treated mice but no histopathological evaluation is given. Cantor and Asofsky do not feel that the effect they observe on thymuses can be attributed to stress which might reduce the cortical cell population as a result of high amounts of corticosteroid. They prefer to suggest that ALS somehow causes an alteration in the proportion of mature cells which are about to leave the thymus to become T cells. It is reasonably supposed that T cells initiate GVH reactions.

Whatever the mechanism involved, these experiments suggest that studies on immunosuppressive agents should take into account changes in the source organs of immunologically reactive cells. From a Correspondent

How Fickle is the Finger of Fate?

IT is sometimes difficult to know how seriously to take those who suggest that the constants of physics are really variables. The numbers game is fun, of course, and is undoubtedly an exercise which ranks among the better kinds of mental gymnastics for preventing premature decay of the grey matter. But is there really any practical point to it? As Noerdlinger points out, in introducing the latest version of this game, "there are grave doubts as to the meaning of saying that any dimensional constant changes, because presumably dimensional constants only express something about the sizes of the standards we use in measuring them". Nevertheless, Noerdlinger goes on to consider the implications of the 2.7 K background radiation for the constancy of Planck's constant h (*Phys. Rev. Lett.*, **30**, 761; 1973).

The latest bout of speculation about the constancy of h seems to have been sparked off by the suggestion of Bahcall and Salpeter that measurements of the dispersion of QSO light by prisms and by gratings could provide a measure of the fickleness of the constant (*Astrophys. J.*, **142**, 1677 ; 1965). Their idea is straightforward enough: because prisms essentially react to the energy of photons whereas gratings measure wavelength and $E=h\nu$, comparison of the two kinds of observation would show up changes in h since the QSO light started on its journey. Experiments to test this possibility have come up with negative results in every case. But still, these experiments have only finite accuracy, so the possibility of a variable h remains. Now Noerdlinger has come up with an alternative test which, while proving equally negative (or equally positive, perhaps, if one's point of view is that universal constants really should be constant throughout time and space), does provide further mental stimulation.

A fundamental requirement of any such contribution to the numbers game is to make enough initial assumptions to ensure that something can be deduced. Noerdlinger's assumptions about the origin of the black-body background are reasonable, although one could quibble over his exact choice of parameters, and do not seriously affect his principal point. Like the QSO light idea, this is quite simple. The Rayleigh-Jeans part of the measured 2.7 K curve gives the present value of kT irrespective of the value of h. On the other hand, the turnover in the spectrum gives an indication of the parameter $(h\nu/kT)$. Treating kT as one parameter, and leaving aside any question that k changes, a measure of h which might be expected to carry some memory of conditions earlier in the evolution of the Universe again seems possible. If h was larger in the past, for example, the spectrum ought to turn over below the points measured. Noerdlinger concludes that $\delta h/h<0.3$ in the interval of redshift $0<z<1,000$ (his upper limit on z corresponds to the assumed time at which the last energy interchange between matter and radiation occurred—the end of the decoupling era).

An interesting possibility remains, however. If the QSO experiments do produce a positive result, the lack of a positive result from the background radiation test might imply that the speed of light varies, that one should not write $(h\nu/kT)$ but rather $(hc/\lambda kT)$, and/or that the 2.7 K radiation is travelling at a speed not equal to c.

By our Cosmology Correspondent

SYSTEMATICS

Hope for Britain's Wildlife

from a Correspondent

On April 11, 12 and 13 the Systematics Association held a symposium in Leicester to consider the changes which are occurring in the numbers and distribution of the principal groups of plants and animals in Britain. This was the first meeting with such a comprehensive cover since the Linnean Society's conference in 1935.

Professor K. Mellanby (Monks Wood Experimental Station and University of Leicester) opened the proceedings with a description of the environmental changes—climatic, population and industrial—with which the flora and fauna have to contend. He also summarized the general conclusions in the final contribution. Almost every speaker had used maps prepared by the Biological Records Centre at Monks Wood. The success of this centre depends on the help and collaboration it receives from many observers and recorders, professional scientists and amateurs alike. Professor Mellanby thought that this practical cooperation between conservationists augurs well for the future, and that it will encourage the scientists from the Nature Conservancy, who are being reorganized into the Institute of Terrestrial Ecology within the Natural Environment Research Council, to continue to direct their research into fields of wildlife conservation.

Dr F. H. Perring, also of Monks Wood, dealt with flowering plants. Some twenty species have disappeared since recording began, and fifty are classified as "endangered", but others, not all introductions, have extended their range considerably. Dr F. Rose (Kings College, London) dealt with bryophytes and Dr L. Hawksworth and Mr B. J. Coppins (Commonwealth Mycological Institute) with lichens. These groups have been used as delicate indicators of atmospheric pollution, and several species are endangered by such factors as tree felling and the general "tidying up" of old buildings and of the countryside generally. Dr W. E. Jones (University of Wales, Bangor) spoke on seaweeds, on which new studies by divers are extending knowledge.

Dr G. B. Corbet (British Museum (Natural History)) said that although six native species of mammal became extinct between Roman times and 1800, the remainder seemed to be holding their own. Dr J. T. R. Sharrock and Mr K. Williamson (British Trust for Ornithology) showed that there has been a net gain of five new species of bird entering Britain every decade since 1950, notwithstanding habitat destruction and human persecution. This was a success story for practical conservation. Dr T. T. Macan (Freshwater Biological Association) and Mr A. Wheeler (British Museum (Natural History)) were both chiefly concerned with the success of introductions of invertebrates and fish to freshwater.

Mr I. Prestt (Department of the Environment) spoke on reptiles and amphibians, each of which have six British species. Several of these are really endangered, and the numbers of others, including the common frog, have decreased. Dr A. South (Sir John Cass College, London) showed that although some snails have suffered, some slugs are well adapted to modern agriculture and are now pests. The spores of pathogenic fungi are widespread according to Dr C. Booth (Commonwealth Mycological Institute, Kew), and records show the conditions which make their presence known (for example, disease outbreaks). Dr D. R. Reid (also from Kew) showed how fungal records are sometimes incomplete because collectors get up too late to see transient forms visible only soon after dawn.

Dr E. Duffey (Monks Wood) showed how records have improved, and the British list has lengthened, as a result of the recent efforts of the Arachnological Society. Only one spider is thought to have disappeared in recent years. Mr J. Heath (also of Monks Wood) described how habitat changes affect butterflies and moths, but showed that recently improvements in distribution often balance disappearances and the situation, generally, is not entirely unfavourable. In the cases of other insects, Mr J. C. Felton (Shell Chemicals) and Mr K. G. V. Smith (British Museum (Natural History)) showed that, in the Hymenoptera and Diptera respectively, the species recorded increase yearly as studies are intensified. Mr Smith indicated that man is deliberately trying to exterminate flies which bite man. Professor Mellanby described how some human ectoparasites are faring; head lice are getting commoner because habitat improvement (longer hair) favours the insects.

The chief impression gained from the Leicester symposium was that although some species and many habitats are endangered by population, affluence, mobility and industrial growth, the position is not nearly as bad as is often suggested. The report of the World Wildlife Fund a few days earlier had given a quite different impression, stating categorically that "nearly a quarter of all Britain's vertebrate animals and plants are threatened" and listing, as seriously endangered species, many which are well known to be flourishing and increasing. At Leicester the speakers were recognized authorities on their groups. Though generally reasonably optimistic, they were in no way complacent, and frequently drew attention to dangers, though they all avoided the hysterical and counterproductive attitude unfortunately characteristic of some "ecologists" and, now, of the World Wildlife Fund.

PALAEOICHTHYOLOGY

Tetrapod Ancestry

from a Correspondent

The lobe-finned fishes (rhipidistians) have long been regarded as the closest relatives of the tetrapods. Jarvik, who has worked on these fishes for almost

Nucleolar Single Stranded DNA

In eukaryotic cells, as in *Escherichia coli*, newly replicated DNA can be extracted in a single stranded form and it has been suggested that replication of nucleolar DNA may be independent of the replication of the bulk of the genomic DNA of eukaryotes. These considerations led Almaric *et al.* to investigate the form of DNA that can be extracted from the nucleoli of ascites tumour cells and, as they report in *Nature New Biology* next Wednesday (May 9), single stranded DNA can indeed be extracted from the nucleoli of these tumour cells with hot phenol and cresol in the presence of para-aminosalicylate.

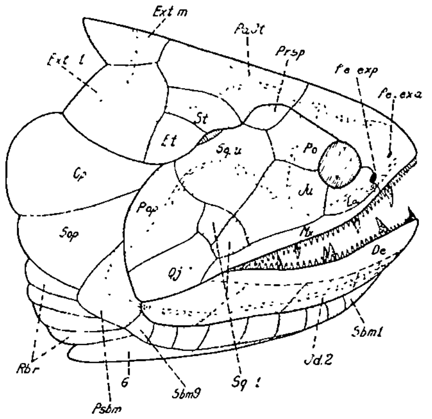
This single stranded nucleolar RNA does not, however, have the properties of newly replicated DNA; it remains constant for long periods of chase in pulse chase experiments and so cannot be a replicative precursor of stable double stranded DNA. Moreover, the base composition and lack of complementarity with ribosomal RNA indicate that this single stranded nucleolar DNA is not related to ribosomal RNA genes in the nucleolus.

The kinetics of labelling, the base composition, the nucleolar localization and the lack of complementarity with ribosomal RNA all indicate that this single stranded nucleolar DNA comprises a discrete portion of the cell genome. Its function remains unknown, but Almaric *et al.* speculate that it may act as a recognition site and have some regulatory function. The existence of single stranded stretches of duplex DNAs with this function was suggested by Crick recently.

forty years, has concluded (*Medd. Grønland*, **187**, 307 pp. ; 1972) that the newts and salamanders (urodeles) have descended from one group of rhipidistians, and the frogs and all other tetrapods, including ourselves, from another.

His study is based on fossils from Greenland and Spitsbergen, where he has collected, notably with the English-Norwegian-Swedish Spitsbergen expedition of 1939 and thirty years later with the French expedition of 1969. Between-times he worked with the Danes in East Greenland. Much of the material described in this article was collected on these expeditions.

Jarvik usefully summarizes his lifework on rhipidistians and the origin of tetrapods. He presents new restorations of the heads of ***Holoptychius***, ***Glyptolepis*** and ***Porolepis*** and gives the first full account of his serial sections through the snout region of *Glyptolepis*. There are also valuable restorations of lower jaws, gill skeletons and shoulder girdles. Jarvik's views on the dual origin of the tetrapods result from highly detailed comparisons between the heads of rhipidistians and living Amphibia. Unfortunately few other forms are known in comparable detail and, because the validity of comparative anatomy depends largely on a knowledge of as great a variety of types as possible, one would like to see similarly profound studies of other forms. Such a survey is, however, beyond the scope of an individual.



Jarvik's restoration of the skull of *Glyptolepis groenlandica* (from *Medd. Grønland*, **187** ; 1972).

Jarvik also deals with another controversy—that of the origin of the vertebrate skull. Although it is generally accepted that the skull is segmental in organization, the idea that it is composed of modified vertebrae (first conceived by Goethe and the "natural philosophers") has not gained many adherents. Nevertheless Jarvik provides stimulating and forceful arguments in support of this view.

In spite of the controversies, the greatest tribute is due to Professor Jarvik and his colleagues in Stockholm who have, through patient and meticulous work carried out since the 1920s, vastly increased knowledge of extinct forms, and the status of the science of palaeontology.

PHYTOCHROME

Membrane Constituent?

from our Phytochemistry Correspondent

PHYTOCHROME is a photochromic protein present in all green plants in which it mediates a wide range of light-initiated activities ranging from rapid movements of leaflets and chloroplasts to longer term effects on metabolism and morphogenesis. Much evidence has accumulated recently that phytochrome is a membrane protein (or at least associates with membranes) and that it regulates the ionic permeability and thus the electrical potential of certain cell membranes. Roux and Yguerabide (*Proc. US Nat. Acad. Sci.*, **70**, 762 ; 1973) now show that purified phytochrome can spontaneously enter and alter the conductance of artificial membranes.

Artificial bilayer membranes were constructed from 7-dehydrocholesterol and oxidized cholesterol across an aperture in a 'Teflon' cup and were stable for about 30 min. Phytochrome purified from oat seedlings (molecular weight about 110,000) was injected in the Pr form into the cup and the conductance was measured while the membrane was sequentially irradiated with 660 nm and 720 nm light. The red light caused a ten-fold drop in membrane conductance which, in the case of oxidized cholesterol membranes, was readily and repeatedly reversible by the far-red light. Boiled, aged or otherwise denatured phytochrome had no effects. The simplest explanation of these results is that the membrane-bound Pfr form of phytochrome induces a higher conductance than the bound Pr form.

The fact that phytochrome is a water-soluble protein with a high content of polar amino-acids has caused many plant biochemists to be reluctant to accept it as a membrane constituent, but Roux and Yguerabide point out that the molecule has an even higher content of nonpolar amino-acids. Thus phytochrome may have considerable regions of nonpolarity which are capable of interacting with the hydrophobic environment of the lipid bilayers, and possibly with intracellular membranes.

Other interesting information of the properties of phytochrome has come from the immunological experiments of Pratt (*Plant Physiol.*, **51**, 203 ; 1973) who has found that purified phytochrome from peas is not immunologically identical to that from oats, rye and barley. Phytochrome from two different cultivars of oats was identical, although that from rye and barley differed slightly from the oat samples. Cundiff and Pratt (*Plant Physiol.*, **51**, 210 ; 1973) have also investigated the immunological behaviour of large and small phytochrome preparations from oats. When phytochrome is carefully isolated, it has a high molecular weight of around 240,000. Another fraction with a molecular weight of 60,000 is often found and this is produced artefactually by protease hydrolysis during extraction. Cundiff and Pratt have found that large phytochrome has at least two antigens which are not present in the small phytochrome. These two antigens cannot be detected spectrophotometrically and apparently one has a higher molecular weight than the small phytochrome fraction. Thus, proteolysis of large phytochrome yields a moiety of 60,000 molecular weight and other moieties, one larger than 60,000 molecular weight, which are not associated with the chromophore.

RNA TUMOUR VIRUS

Leukosis Agent

from our Cell Biology Correspondent

FOR some time the idea that avian leukosis viruses may in essence be deletion mutants of non-defective avian sarcoma viruses has been going the rounds without any definitive experiments being attempted. A considerable body of circumstantial evidence in support of the idea has been accumulated, but the only way to test whether a virus can cause leukosis in chicks is to inoculate birds and see how they die. There are not that many laboratories equipped for such experiments, but at Houghton Poultry Research Station, Biggs and Milne have recently tested the oncogenicity of two mutants of Schmidt Ruppin Rous sarcoma virus isolated by Graf *et al.* As Graf and his colleagues had shown, these two mutant strains of Rous sarcoma virus have lost the ability to transform chick fibroblasts and they also have all the other *in vitro* properties of avian leukosis viruses. But do they have the ability to cause leukosis in chicks? According to Biggs *et al.* (*J. Gen. Virol.*, **18**, 399 ; 1973) they do.

Biggs *et al.* inoculated day-old chicks from flocks of strains apparently free of leukosis and sarcoma viruses of subgroups A, B, C, and D with Graf's mutants which belong to subgroups A and D. One of the strains of chicks used is particularly susceptible to injection by sarcoma virus infection and the other is very susceptible to leukosis virus. The inoculated birds were then watched; several contracted Marek's disease and were lost from the experiment; none developed sarcomas, which is to be expected because Graf's mutants are non-transforming. Up to 50 per cent of the birds of the strains susceptible to leukosis virus developed either erythroid or lymphoid leukosis as a result of inoculation with the non-transforming Rous sarcoma mutants. This striking result indicates either that the mutant virus genome has the capacity to induce leukosis or it is able to recombine with an endogenous, latent leukosis virus genome in the chicks. But in any event the mutant viruses can be called leukosis viruses because they induce leukosis either directly or indirectly. Furthermore these experiments establish that more than one gene in avian leukosis/sarcoma viruses can cause neoplasia.

If all chick cells did not inherit the genetic information required to specify an avian leukosis virus (using that term in its taxonomic rather than pathological sense), the interpretation of the experiments of Biggs *et al.* would be simpler and more clear cut. But the fact is that all chicks do apparently inherit a DNA provirus of a leukosis virus; where they differ is in the extent to which this endogenous viral genome is expressed. As Hayward and Hanafusa (*J. Virol.*, **11**, 157; 1973) report, chick cells which contain the group specific antigen of the avian RNA tumour viruses and the helper factor for replication of infectious BH.RSV contain between three and forty copies per cell of viral RNA which is apparently transcribed from less than about 50 per cent of the endogenous leukosis virus genome. By contrast, cells which lack the gs antigen and helper factor do not contain any detectable viral RNA but they do contain viral DNA. Further, cells which support the replication of an exogenous leukosis virus RAV-2 contain 3,000 to 4,000 copies of complete transcripts of the viral genome.

All these data are consonant with the dogma that all chick cells inherit a DNA provirus of an avian RNA "leukosis" virus and that the extent to which the endogenous genome is expressed is controlled by other cellular genes. Whether the endogenous virus has any oncogenic potential is an important question that has yet to be answered.

MAGNETIC RESONANCE

EPR in a Superconductor

from a Correspondent

THE first unequivocal observation of electron paramagnetic resonance (EPR) of a localized moment in a superconductor has been claimed by Rettori *et al.* of the University of California (*Phys. Rev. Lett.*, **30**, 437; 1973). The experiments were performed on the intermetallic compound $LaRu_2$ containing various concentrations of gadolinium. It is the Gd atoms which provide the local moments that are the source of the resonance signal.

The magnetic impurities reduce the tendency to superconductivity of the $LaRu_2$ as they act as an unpairing mechanism on the paired electrons, the so-called Cooper pairs, which are responsible for the superconductivity. For gadolinium concentrations which are not too high, however, the alloy remains a superconductor. The applied magnetic field needed to perform the resonance experiment also tends to destroy the superconductivity. But in spite of the destructive mechanisms, the alloys used by Rettori *et al.* were superconducting in the presence of the resonance field at temperatures less than about 3 K and, below 2.8 K, they were able to observe X band EPR in the superconducting state. EPR in the normal state was also observed at temperatures greater than 3.2 K.

From the results of the experiments it was possible to obtain estimates of the exchange and spin-orbit scattering rates. These rates determine how an excited Gd atom loses its energy. Initially the energy is lost to the conduction electrons by exchange scattering and then the conduction electrons lose the energy to the lattice by means of their coupling through the spin-orbit interaction. If the exchange scattering rate is higher than the spin-orbit scattering rate a "bottleneck" is formed because energy is piled up in the conduction electrons. It is rather like a tank which is being filled and emptied at the same time. If the filling rate is higher than the emptying rate then the tank becomes full and any further filling is prevented. For the $LaRu_2$:Gd system in the temperature range used in the experiments no bottleneck was observed in either the normal or superconducting states. But recent theory (K. Maki, unpublished) predicts an increasing exchange rate and a decreasing spin-orbit rate on going from the normal to the superconducting state. Thus, now that EPR has been observed, there is the exciting possibility that bottlenecked systems can be measured, and it should then be possible to determine directly the dynamics of the conduction electron spins in the superconducting state by making use of the changing EPR-bottleneck conditions.

GEOCHEMISTRY

Pulling Together

from a Correspondent

ENVIRONMENTAL biogeochemistry, the subject of a symposium held in Logan, Utah, from March 22 to 24, is more an area of common interest than a new discipline. The emphasis throughout the meeting was on the understanding of environmental processes involving chemistry and microbiology and the connexion between these studies and the geological record. Perhaps the principal achievement of the conference was that it highlighted the considerable current activity in the study of the interaction between microorganisms and inorganic materials. Microbiologists, chemists, soil chemists and so on often have slight knowledge of each other's disciplines, however, and it was in bringing people together to effect a cross fertilization of ideas and techniques that the confer-

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Maturation of Ribosomal RNA

IN *Nature New Biology* next Wednesday (May 9) Chang and Irr report a series of experiments in which they have analysed the synthesis and maturation of ribosomal RNAs in stringent and relaxed strains of *Escherichia coli* starved for leucine. Their data indicate that in both strains ribosomal RNA genes are transcribed to yield precursor ribosomal RNAs, albeit in different amounts. Furthermore in both stringent and relaxed strains of *E. coli* in conditions of amino-acid starvation the maturation of the ribosomal precursor RNA is blocked at its terminal stages so that precursor 16S and precursor 23S ribosomal RNAs are not converted to mature 16S and mature 23S ribosomal RNAs. In other words "accumulation of rRNA precursors is not an artificial situation brought on by starvation of a mutant which has lost the ability to regulate the synthesis of RNA but, rather, a phenomenon which occurs in both rel^+ and rel^- bacteria during leucine starvation".

But why does amino-acid starvation, apart from any effect on RNA synthesis, block the maturation of ribosomal RNA precursors at some late stage in the maturation process? Chang and Irr suggest that certain ribosomal proteins are required to alter the configuration of precursor ribosomal RNAs and therefore protect them from complete degradation but make them available for the precise cleavages that result in correct maturation. If the pools of these ribosomal proteins are exhausted during amino-acid starvation, maturation will not go to completion. In short, Chang and Irr believe that during amino-acid starvation precursor ribosomal RNAs accumulate in *E. coli* because the supply of ribosomal proteins that are essential for maturation becomes limiting.

ence has made its most valuable contribution.

Interesting studies reported included those described by Dr T. D. Brock (University of Wisconsin) of sulphur bacteria living at 95° C and *p*H 2 in hot springs of Yellowstone National Park; by Dr W. E. Krumbein (Biologische Anstalt, Helgoland) of lichens which bring about the formation of "desert varnish" on the exposed rock surfaces; by Dr H. L. Ehrlich (Rensselaer Polytechnic Institute) of the microorganisms involved in the formation and dissolution of ferromanganese oxide nodules on the ocean floors; and by Dr J. V. Beck (Brigham Young University) of those bacteria (*Thiobacillus oxidans*) responsible for accelerated leaching of copper from mine tips. Some environments were discussed at considerable length, especially those involving stromatolite formation by blue-green algae. Thus, Dr S. Golubic (Boston University) gave a fascinating account of the sequence of events taking place on the coast near Abu Dhabi in the Persian Gulf. These modern environments provide invaluable insights into the fossil record for geologists, geochemists and palaeontologists. Dr Golubic's contemporary stromatolites serve as a guide to the understanding of the Precambrian stromatolites described in talks by Dr J. W. Schopf (University of California, Los Angeles), Dr S. M. Awramik (Harvard University) and Professor P. E. Cloud (University of California, San Diego).

Other studies described by participants included investigations of aquatic environments in the Everglades of Florida, the Severn Estuary and certain lakes in the British Lake District and North America, and the Arctic Seas. Mathematical models built up in small computers are now in common use by geochemists and ecologists for the study of element, energy and mass balances within individual organisms and complete ecosystems. The kinetic models of Dr R. A. Berner (Yale University) for the early diagenesis of nitrogen, phosphorus and sulphur in anoxic marine sediments were described by Dr I. Kaplan (University of California, Los Angeles) and other speakers as helpful though incomplete treatments of very complex situations. In this context it is clear that pollution studies have to be seen in relation to the natural fate and the natural variability of elements and compounds in environments. This was illustrated by the work of the group at the University of Bristol (described by Dr G. Eglinton) on the short-term fate of selected biolipids (oleic acid, phytol and cholesterol) and of DDT in estuarine and lacustrine sediments.

The meeting concluded with a field trip to the algal mats at the north shore of the Great Salt Lake. Such a trip should be an integral part of any future conference, for it provides a focus for first-hand joint experience and discussion of a particular ecosystem. The interfaces between biosphere and geosphere need just this type of interdisciplinary attack. There was enthusiastic support for the organization of another conference in two years time with participation extended to include natural product chemists, biochemists, kineticists and mathematicians so that the problems can be more thoroughly discussed.

MICROWAVES

Expanding above 10 GHz

from a Correspondent

A FEATURE of radio research and development in recent years has been the increasing interest in propagation at frequencies greater than about 10 GHz. The large bandwidth available at these frequencies is particularly attractive in terrestrial and space communication systems for additional channels of information. Absorption and scattering in the lower atmosphere also becomes more important, however, as the frequency increases about 10 GHz. Consequently, practical exploitation of this part of the spectrum requires extensive basic research on wave propagation. This dual theme of the development of communication systems and the limitations imposed by meteorological factors was emphasized at the international conference held between April 10 and 13 at the Institution of Electrical Engineers.

In his opening address Mr J. H. H. Merriman, deputy president of the IEE, pointed out that the upper limit of radiofrequencies discussed within the context of international control was increasing by a factor of about ten every decade. Frequencies up to nearly 300 GHz (wavelengths down to 1 mm) have provisionally been allocated for various requirements. A topic of particular interest is the reliability of terrestrial radio relay systems above 10 GHz, and the prediction of their performance from radio meteorological data. Very heavy rain can cause serious fading and this effect increases steadily with increasing frequency. Contributions from authors at the Radio and Space Research Station of the Science Research Council and from the Post Office Research Department described a comprehensive experiment to investigate this problem. Simultaneous measurements of rainfall structure and of fading on several links at frequencies of 11, 20 and 37 GHz are in progress in Suffolk. From this work it is hoped to predict the optimum lengths and spacings of microwave links to avoid the worst effects of heavy rain. Members of the Post Office Research Department also discussed the fading ("multipath propagation") which can occasionally occur in clear conditions on terrestrial links as a result of abnormal refraction. These multipath effects are particularly important in view

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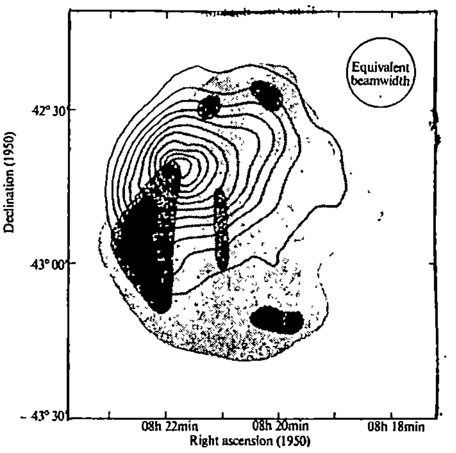
Puppis A Mapped by Copernicus

THE supernova remnant Puppis A has been mapped in the energy range 0.5 to 1.5 keV with better resolution than in any previous observations. The X-ray emission is found by Zarnecki and his colleagues to be strongly peaked and concentrated chiefly in the north-east quadrant of the radio shell (see next Monday's *Nature Physical Science*, May 7).

Previously, one-dimensional scans of Puppis A and rocket borne collimated proportional counters have shown that the angular size of the X-ray emitting region is less than 0.5 arc degree. The source is one of the brightest known at X-ray frequencies less than 2 keV and the X-ray spectrum is best fitted by a combination of thermal bremsstrahlung and line emission from a plasma at 4×10^6 K. The latest map, however, provides the first data obtained with high spatial resolution in two dimensions.

The data are not consistent with a simple point source and disk model of the source, and the X-ray emission is contained within the corresponding radio shell. A compact object may remain inside the remnant, but the evidence is not conclusive in this respect. On the whole, Zarnecki *et al.* feel that the observed X-ray and radio features can be adequately explained by existing supernova models.



Contour map of Puppis A at soft X-ray frequencies (shaded). Solid contours are radio observations, after Milne (*Austral. J. Phys.*, **24**, 429; 1971).

of the wide bandwidths now required for various kinds of data transmission.

Mr R. W. Wilson and Mrs W. L. Manmel (Bell Telephone Laboratories) and Professor D. B. Hodge (Ohio State University) presented data from spaced-receiver measurements of Earth-space attenuation. At a frequency of 16 GHz, receiver separations of 10–15 km in a switched-path network give a significant improvement in reliability for the locations used in the United States. As the results presented by Dr P. G. Davies (Radio and Space Research Station) showed, however, there is a considerable variability from year to year at a given location and also from one climate to another. Results from RSRS illustrated that South-east England has an excellent climate from the point of view of reliable space communication. Even frequencies as high as 100 GHz may be useful there for short-hop terrestrial paths or Earth-space links in or near the vertical.

Several contributors presented results of radiometric measurements of thermal emission noise from the troposphere. This technique affords a simple method of deriving attenuation values of up to 10–12 dB on Earth-space links. Radiometers have also been used to study thermal noise from the local environment around the site of an Earth station. The results of Mr F. V. C. Mendis and Dr T. Pratt (University of Birmingham) and of Mr D. M. Knox, Mr R. G. Howell and Mr J. A. McLeod (Post Office), at 9 and 17 GHz respectively, gave values for the noise radiated from buildings, foliage, and various types of ground surface. In general, however, the contribution from the sky noise is more important than that from local ground-based sources.

For reasons of economy of space in the microwave spectrum, sharing of frequencies by transmissions using orthogonal polarizations has been proposed. Contributions from the universities of Bradford and Essex and from the Post Office dealt with theoretical and experimental work on this topic. Although it seems likely that distortion of the plane of polarization by the constituents of the troposphere will not prove serious on short links at frequencies of about 20 GHz, further work is required at a range of frequencies and in various weather conditions. Studies of the shape and orientation of raindrops are also necessary to extend the theory of cross-polarization. This is one example of a general requirement for basic radio meteorological data to assist future developments in communications. In spite of much important work related to forecasting requirements in meteorology, remarkably little is known about the varying structure of heavy rain over distances important in microwave communication, of the order of a kilometre.

Several contributors demonstrated that, quite apart from communication interests, frequencies greater than 10 GHz have special advantages in remote probing of tropospheric structure. For example, anomalous results, difficult to explain by existing theories, were shown in measurements of atmospheric emission at 15 GHz by Dr M. S. Reid and Mr T. J. Lockhart (California Institute of Technology and Air Associates). Similarly, at 85–118 GHz, the results of Dr C. J. Gibbins, Mr A. C. Gordon-Smith and Dr D. L. Croom (Radio and Space Research Station) showed a greater variability in emission noise than expected on the basis of data on the water vapour content and temperature structure. There are probably features of atmospheric structure (such as molecular complexes and the effect of minor constituents) which are not yet understood. Frequencies greater than 10 GHz will, in this respect, prove a happy hunting ground for radio physicists.

NUCLEAR PHYSICS

Recoil and Transfer

from a Correspondent

VERY many investigations have established the one-nucleon transfer reaction as a powerful way of investigating the single-particle character of nuclear states. Most of this work has used incident particles consisting of rather few nucleons, in particular the familiar (d,p) and (d,n) reactions, and also reactions initiated by tritons, helions and alpha particles.

In recent years, however, heavy ions have come into their own now that they can be accelerated to energies sufficiently high to surmount the Coulomb barrier of the nucleus and interact with the nuclear field. These reactions have several features that make it useful to study their properties in detail. In par-

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Time of the Corsica-Sardinia Rotation

THERE is now convincing evidence that Corsica and Sardinia have rotated in an anticlockwise direction from positions adjacent to southern France. Such a rotation is consistent with palaeomagnetic data from Corsican Permo-Carboniferous rocks and from Permian and Oligo-Miocene rocks of Sardinia. Moreover, Corsica makes a good fit with France along the 1,000 m isobath, enabling Permo-Carboniferous outcrops in Corsica to be matched with similar outcrops in Provence and permitting the alignment of offshore magnetic anomalies thought to result from andesitic volcanoes.

But there is perhaps less agreement on precisely when the rotation took place, estimates of the time of rotation having ranged from Triassic to Miocene. Some years ago, Nairn and Westphal (*Palaeogeog., Palaeoclimatol., Palaeoecol.*, **5**, 179; 1968), for example, suggested that the rotation may have occurred during the Mesozoic, although more recently, and in the light of additional data, Alvarez (*Nature Physical Science*, **235**, 103; 1972) proposed a Tertiary rotation. More specifically, Alvarez adduced evidence showing that Corsica-Sardinia could not have separated from France before 11 or 12 million years ago. The completion of the rotation, on the other hand, was less easily timed, although the presence under the Ligurian Sea floor of salt domes derived from upper Miocene evaporites led Alvarez to conclude that rotation must have ended early enough to allow the evaporite deposition during part of the Messinian period, 7–5 million years ago.

In next Monday's *Nature Physical Science* (May 7), Alvarez reports a further attempt to determine the time of completion of the rotation, based on the palaeomagnetism of Plio-Pleistocene basalts from north-west Sardinia. Two distinct generations of basalt (designated β_1 and β_2) were represented in the collection examined. Unfortunately, because of dating problems, absolute ages are available for neither set; but the younger generation (β_2) are associated with cinder cones almost unaffected by erosion and must therefore be late Pleistocene or Holocene, and the older set (β_1) are regarded as Pliocene or possibly late Miocene.

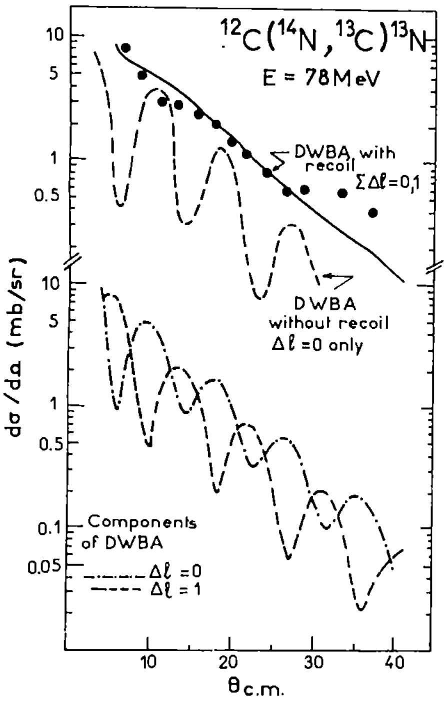
The mean palaeomagnetic direction from the β_2 samples (declination 350°, inclination +51°) agrees closely with that (353°, +53°) recently obtained by Bobier and Coulon (*CR Acad. Sci. Paris*, **270**, 1434; 1970). Both directions, and all other mean palaeomagnetic directions from Sardinian basalts which are definitely post-Miocene, cluster around the present field direction, showing that the rotation must have been completed before the end of the late Miocene (about 10 million years ago). Because samples from one of the three (older) β_1 sites turned out to have an intermediate direction, Alvarez calculated no β_1 mean palaeomagnetic direction from his data alone. The overall mean (354°, +56°) calculated from Alvarez's and other sources of β_1 data is, however, very close to that (352°, +48°) obtained from all β_2 data, showing that, taken at face value and neglecting errors, the (older) β_1 mean is actually closer to the present field direction (0°, +60°) than is the (younger) β_2 mean. Thus, as Alvarez points out, the β_1 data dc not confirm the suggestion from Manzoni *et al.* (*Giornale di Geologia*, **38**, 5; 1972) that the β_1 basalts predate the completion of rotation—both generations of basalt seem to postdate it.

ticular, they often show semi-classical characteristics that simplify the analysis of the cross-sections, and also the heavy ions bring in high angular momenta that make it possible to excite states of high spin. Furthermore, these reactions often show marked selectivity in that they tend preferentially to populate states of a particularly simple structure.

The distorted wave Born approximation (DWBA) formalism has been used with great success to analyse the (d,p) and similar reactions; it has yielded important information on nuclear structure, particularly the spectroscopic factors. This theory has naturally also been applied to one-nucleon transfer reactions between heavy ions and it has been found to give excellent results in some cases but not in others.

A particularly notable discrepancy is found for reactions between light nuclei. These have cross-sections that fall off steadily with increasing angle, whereas the DWBA predicts angular distributions showing a marked oscillatory structure. The reason for this is that in conventional DWBA calculations the nuclear recoil is neglected, and this is justified if the mass of the incident particle is much less than that of the target nucleus. In the case of reactions between heavy ions these masses are comparable and recoil can no longer be ignored.

Examination of the angular momentum conservation relations in the reactions shows that if recoil is neglected the change Δl in the orbital angular momentum of the transferred particle must be zero, whereas if recoil is taken into account Δl may be 0 or 1.



Differential cross-section for a one-nucleon heavy ion transfer reaction compared with DWBA calculations. The upper dashed curve shows the DWBA calculations without recoil ($\Delta l=0$ only) and the two lower curves the DWBA calculations with recoil ($\Delta l=0$ and 1). The sum of these two lower curves is the full curve, which agree well with the experimental data.

The inclusion of recoil effects makes the calculations much more complicated, but it still remains within the capacity of fast computers. A program including recoil effects has recently been written and DeVries and Kubo (*Phys. Rev. Lett.*, **30**, 325; 1973) have used it to investigate the importance of these effects. They find, as shown in the diagram, that the $\Delta l=0$ and $\Delta l=1$ contributions to the reaction amplitude both oscillate as a function of reaction angle, but that the oscillations are out of phase so that their sum varies smoothly with angle, just like the experimental data. This shows very clearly that recoil effects must be included in DWBA calculations of nucleon transfer between heavy ions of comparable mass.

The calculations also give the product of the two spectroscopic factors for the transition, and this is close to the theoretical value of Cohen and Kurath. Thus the DWBA theory is able to give a good account of both the angular distribution and the absolute magnitude of the cross-section for single-nucleon transfer between heavy ions. It now seems likely, subject to further tests, that these reactions can be used with some confidence to determine unknown spectroscopic factors and will thus become an important tool for the determination of nuclear structure.

Ultrasonic Velocities in Troodos (Cyprus) Rocks

ALTHOUGH the idea that the Troodos Igneous Complex of Cyprus is an upthrust segment of (Mesozoic) seafloor has been around now for about a decade, the evidence to settle the matter with certainty has still not been obtained. Nevertheless, most, if not all, of the relevant geophysical data currently available are consistent with the seafloor interpretation, suggesting that any refutation of the idea in the future is unlikely.

In the meantime, the search for that vital piece of evidence goes on, largely by geophysical means. One of the obvious approaches to the problem is the measurement of seismic velocities, the hope being that the velocity structure of the Troodos Massif will prove to correspond with the unique structure of the present ocean floor rather than with the variable structures of continents. Matthews *et al.* (*Nature Physical Science*, **231**, 200; 1971), Lort and Matthews (*Geophys. J.*, **27**, 383; 1972) and Khan *et al.* (*Nature Physical Science*, **238**, 134; 1972) have all made seismic determinations in the field; but through no fault of these workers, all these investigations have been less than ideal. One of the principal difficulties is that, being upthrust, the Troodos Complex is no longer subject to the confining pressures typical of present or ancient ocean floor *in situ*, so that seismic velocities can hardly be expected to correspond directly. In addition, fracturing in the Troodos units has almost certainly reduced seismic velocities from those applicable at ocean floor depths.

In next Monday's *Nature Physical Science* (May 7), Poster reports attempts to solve these problems by measuring velocities in Troodos samples under pressure in the laboratory. He has determined ultrasonic velocities under hydrostatic pressures of up to 2 kbar and at room temperature for nineteen rock samples representative of each of the principal Troodos units. Typically, velocities increase rapidly up to pressures of about 1 kbar and thereafter remain relatively constant, the initial increase being most marked for the pillow lavas and rocks from the sheeted intrusive complex. But even at atmospheric pressure, velocities were higher than those measured in the field, presumably because of the relative lack of fracturing in the smaller laboratory samples. This velocity difference was greatest in rocks of the plutonic complex (gabbro and ultramafics).

With the exception of the ultramafics, the velocities measured in the laboratory increased with downward stratigraphic order, encouraging Poster to compare the velocity structure with that of "normal" oceanic crust (assuming a purely confining pressure to be exerted on the top of layer 2). At the relevant pressures of 0.5–1.0 kbar, the pillow lava velocities were lower than, and the velocities in the sheeted intrusive complex were generally higher than, the average for oceanic layer 2 (5.1 km s^{-1}). Thus the basal group of the sheeted intrusive complex, comprising screens of pillow lavas with diabase dykes and thought to correspond with layer 2, could easily produce the mean velocity of layer 2 and the wide range of velocities observed in it. Stratigraphically below, measured velocities then increased progressively to typical layer 3 velocities for the fresh gabbros.

The new velocity data thus generally support the view that the Troodos Complex began as oceanic crust, the only anomaly being the fact that the ultramafics have lower velocities at all pressures than the gabbros which lie stratigraphically above. Poster's explanation for this discrepancy is based on his discovery that seven of the ten ultramafics examined were at least 75 per cent serpentinized.

Experiments on Polishing of Diamond

J. WILKS
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Polished diamond surfaces have unusual properties because polishing of the brittle material generally proceeds by mechanical chipping.

Diamonds are shaped and polished to produce gem stones by methods which have remained essentially unchanged for several hundred years. Yet it is only recently that the physical basis of these operations has been studied, and their unusual features fully appreciated. This article outlines the principal features of the polishing process and describes some recent experiments which illustrate the very characteristic nature both of the process itself and the polished surfaces produced. We shall show that these perhaps unique properties result from the very hard and brittle nature of the diamond.

The usual method of polishing a diamond is to hold it either against the face of a rotating cast-iron wheel (or scaife) charged with a suspension of fine diamond powder in a light oil, or against the face of a wheel in which diamond powder is bonded into a metal matrix. One of the most striking features of this method is that if a cube face is prepared on the diamond, it turns out that the rate of removal of material during polishing with a bonded wheel in a direction parallel to a cube axis is about 100 times greater than if the same face is polished in a direction at 45° to a cube axis. Similar effects are observed on other faces, and even greater differences are evident when polishing on a cast-iron scaife, as the more resistant directions of the diamond tend to knock the powder out of the scaife.

In preparing a gem stone from a rough diamond it is necessary to remove a certain amount of material to position the facets before polishing them. Another unusual feature is that this removal of material is effected in almost the same way as polishing, except that larger sized diamond powder is used to remove material quickly. For most materials, polishing and the removal of material by abrasion are quite different processes. Removal of material is generally achieved by the use of an abrasive powder in what is essentially a cutting process; the powder is harder than the specimen, and it either gouges out material, or sets up stresses which lead to cracking after it has moved on. By contrast, polishing is generally a smearing process, brought about by rubbing with a second material to produce high temperatures in the small regions of true contact between the asperities on the two surfaces; the high temperatures result in local melting of the asperities, which are then smoothed out. It follows that to polish a specimen, we must use polishing material with a higher melting point, so that on rubbing, the specimen melts and flows first. For further details of the general features of abrasion and polishing, see for example, Bowden and Tabor[1] and Cottrell[2].

In view of all this, one would expect to encounter difficulties both in removing material from diamonds and in polishing them. As far as the removal of material is concerned, there is no harder substance than diamond which can be used to produce abrasive cutting. In rubbing operations, the high thermal conductivity of diamond will tend to reduce the temperature of the hot spots at the areas of local contact, whereas high temperatures are required to effect even the conversion of diamond to graphite. Thus, conditions seem unfavourable for both abrasion and polishing, but in fact the traditional methods used by the diamond industry abrade and polish diamond quite successfully. The rate of abrasion is relatively low, but a high polish can readily be obtained, and a diamond surface may be worked to the standard of a good optical flat[3].

The first scientific experiments on the polishing process were made more than 50 years ago by Tolkowsky[4], who proposed that both abrasion and polishing proceed by mechanical chipping on a microscopic scale. This chipping will be controlled by the lie of the cleavage planes relative to the direction of abrasion, and will therefore occur more readily in some directions than others, thus giving rise to very considerable differences in rates of abrasion. To indicate the geometrical arrangement of the cleavage planes, Tolkowsky made use of a model of diamond built up from small identical octahedral and tetrahedral shaped blocks, and thus explained the variations of the hardness on both the cube and the dodecahedron planes[4,5].

Tolkowsky was principally interested in practical applications and his conclusions about how to polish and abrade diamonds soon found their way into the diamond trade, as did his other research on the best way to lay out the facets of a diamond so as to produce the well known brilliant cut. On the other hand, his account of the underlying mechanism was not widely published, and attracted little attention. His treatment was criticized on the grounds that diamond is certainly not built up of equally sized elementary blocks as in his model, but the essential function of the model is to indicate the positions of the cleavage planes—and this it does correctly. It now turns out, as the result of various experiments on diamond over the past 15 years, together with increasing knowledge of the nature of brittle materials, that Tolkowsky's views are essentially correct.

One of the crucial features of the above experiment observed but not stressed by Tolkowsky[4], is that the rate of removal of material is proportional to the total number of revolutions of the polishing scaife but does not depend on its speed. This result, confirmed by Wilks and Wilks[5,6], shows that thermally activated processes play no significant part in the removal of material. The rubbing together of two materials produces heating at the areas of true local contact, and this excess temperature increases rapidly with the speed of rubbing[1]. The fact that the abrasion per revolution does not increase with rising speed of rubbing clearly demonstrates that local hot spots play no significant part in the wear process. In particular, it rules out the possibility that the diamond is worn away by either burning or conversion to graphite, as suggested by Seal[7]. On the other hand, one would expect the same amount of material to be removed by each revolution of the wheel if the abrasion proceeds by a mechanical chipping or cleavage process.

The conditions necessary for materials to fail by chipping or brittle fracture have been discussed by several authors, for example Kelly[8]. Kelly, Tyson and Cottrell[9] have considered whether an ideal crystal free of imperfections will fail plastically by shear or by brittle fracture under tension. They show that for face centred metals such as copper, gold and silver, the stress required to produce fracture is about 30 times greater than that to produce shear, and that failure always occurs by plastic deformation rather than cleavage. On the other hand, the calculations show that, of the substances considered, sodium chloride and diamond are most likely to fail by brittle fracture. The critical stresses for shear and cleavage in sodium chloride and diamond are approximately equal, but the calculations are not sufficiently refined to indicate which process is preferred. These calculations are, however, for ideal crystals and the essential question for real materials is whether the presence and motion of dislocations will permit plastic flow before fracture.

Dislocations in diamond have been observed by Evans[10] who prepared specimens of diamond by etching, and viewed the dislocations directly in the transmission electron microscope. They are also clearly delineated by the X-ray topograph techniques of Frank and Lang[11]. Calculations mentioned by Evans[12] suggest that the movement of the dislocations will be inhibited by the necessity of breaking carbon-carbon bonds. Their motion has been studied experimentally by Evans and Wild[12,13] who took specimens of diamond in the form of thin slabs, loaded them in the middle, and observed whether they failed by fracture or plastic deformation. They found that at low temperatures the beam always failed by fracture, but that an increasing amount of plastic flow was observed as the temperature exceeded 1,500° C. Hence dislocations in diamond may be quite mobile, but at room temperature are held back by obstacles which can only be overcome by considerable thermal activation. As the rates of abrasion and polish in the experiments referred to above were independent of the temperature of local hot spots, one concludes that the abrasion was not accompanied by any thermally activated motion of dislocations.

It is sometimes suggested that plastic flow in diamond may be produced by conditions of intense local pressure. The effect of pressure on brittle materials is sometimes surprising, for example it is possible to compress a slab of brittle material, such as rock salt, to half its initial thickness without producing cracking[14], and Howes and Tolansky[15] have argued that the ring cracks produced by indenting diamond with steel spheres show evidence of plastic flow. Later studies by Lawn and Komatsu[16] of the region round such a crack, using X-ray topography, show no sign of plastic deformation, and Frank and Lawn[17] have since shown that the cracks may be described in terms of fracture processes.

We conclude that the mechanism of abrasion is one of mechanical chipping. This conclusion is supported by a study of the area around an abrasion mark, by Frank, Lawn, Lang and Wilks[18] using X-ray topography, which shows that the state of strain in the surface is consistent with stresses set up by fracture processes and not by plastic flow. We believe that their is no convincing evidence for the motion of dislocations in any of the relevant abrasion and polishing experiments, although claims to have observed plastic flow in diamond at room temperature have been made by Brookes[19] and by Gane and Cox[20]. Brooks indented a diamond surface with a Knoop indenter made of diamond and claimed that the form of the indentation indicated that plastic flow had occurred. A careful study of Brookes's indentations, however, show clear indications of brittle fracture (Fig. 1), which appear to have been obscured in the earlier micrographs. The observations of Gane and Cox were based on the study of thin diamond wedges pressed against each other, but their evidence is at best inconclusive. Even under these rather specialized conditions, there is no clear evidence that plastic flow and motion of dislocations took place.

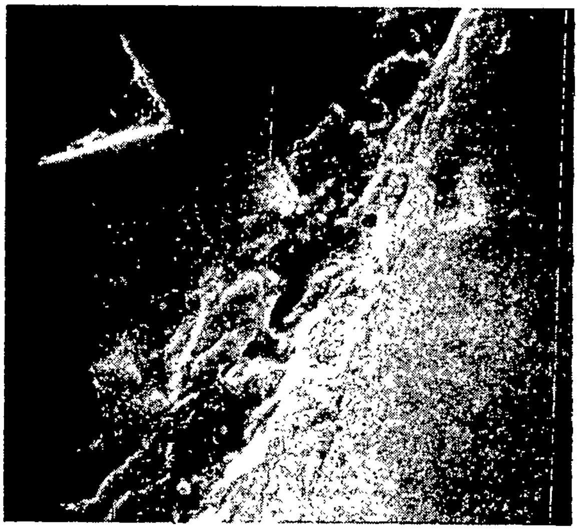

Fig. 1 Scanning electron microscope micrograph of a diamond surface indented with a Knoop indenter (× 8,400).

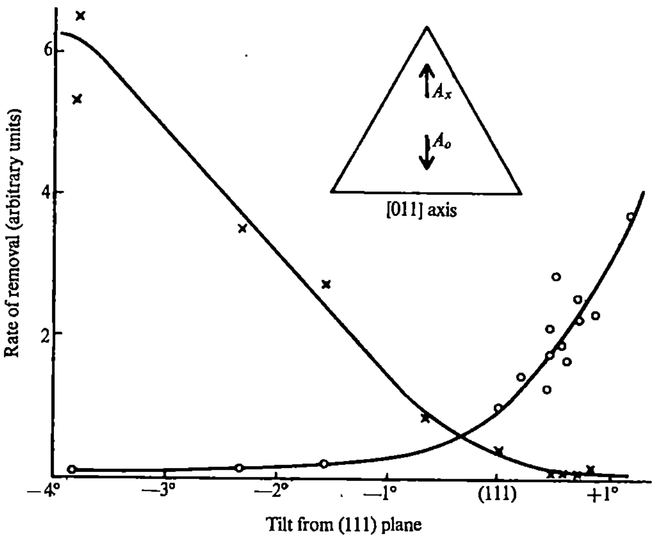

Fig. 2 The relative rates of removal of material by grinding on surfaces near to an octahedron plane. The symbols ○ and × correspond to the directions shown in the inset.

We shall now describe three sets of experiments which confirm the fracture process and illustrates the unusual properties of polished diamond surfaces. In the first set a simple micro-abrasion tester[21] was used to study the variation of the rate of removal of material—in short the hardness of the diamond—with the orientation of the surface and the direction of polish. The hardness is often extremely sensitive to any change in the orientation of the facet being abraded. For example, Fig. 2 shows the relative rates of removal of material on surfaces obtained by tilting an octahedron plane about the (011) axis indicated in the sketch plan. The crosses show the rate of removal of material for abrasion in the direction A_x, and the circles the rate in the direction A_0. We see that on a true octahedron plane the rate of removal in the direction A_0 is more than twice that in the direction A_x, but that a tilt of only about 1° is sufficient to reverse the relative hardness.

These orientation effects are not explained by Tolkowsky's treatment, which also has difficulty in discussing the octahedron face. The variation in rates of abrasion on cube and dodecahedron faces is explained by the lie of the cleavage planes which results in the production of a different structure by abrasion in different directions. As the cleavage planes run parallel to an octahedron face, the treatment seems to predict a uniform surface with no easy and hard directions of abrasion, in contrast with the measurements. To explain this point, we must consider the behaviour of tilted octahedron facets.

A diamond surface which has been polished by a process of mechanical chipping will not be atomatically flat but must consist of hills and valleys bounded by an irregular arrangement of cleavage planes as shown very schematically in Fig. 3*A*. It follows that abrasion proceeds by the removal of material from the tops or sides of the microhills. If Fig. 3*A* represents a section through an octahedron face, parallel to the directions A_x and A_o, the lie of the cleavage planes is such that the most likely sites for the removal of material are the edges *a* and *b* (ref. 5). It also follows that the easiest direction of abrasion is A_o, and that most of the material is then removed from the edges *b*. If, on the other hand, a face is prepared inclined to the octahedron face by a small angle, the lie of the cleavage planes remains the same, so the structure of the microhills is as shown schematically in Fig. 3*B*. Topographically, the abrading wheel must be presented with an increased number of *b* type edges, and the hardness correspondingly reduced. Similarly a tilt in the opposite direction increases the hardness.

One also expects that the change in hardness with tilt may be very rapid. If the linear dimensions of the microhills are all of the same order of magnitude *h*, the average distance between hills in contact with the abrading wheel will be much larger because the areas of real contact are less than the nominal areas by factors of order 10^4. Thus the distance between the hills may be of the order of 100 *h*, so that a tilt of 1° would be sufficient to change the topography of Fig. 3*A* to that of Fig. 3*B*, and thus approximately double the number of sites of easy abrasion. Similar arguments may be applied to account for the hardness of both true and tilted cube and dodecahedron faces. All the predictions that can be made from the model are in accord with experiment[5].

We have also made studies of the jagged nature of polished diamond surfaces, implied by our abrasion experiments, by observing the friction of diamond sliding on diamond in air. Some time ago Seal[7] showed that the friction of a polished cube surface varies with the azimuthal angle of sliding, with a four-fold symmetry. This result is at first surprising, as Bowden and Hanwell[22] have shown that diamond surfaces in air are covered by a strongly adsorbed film which reduces the friction by an order of magnitude below its value for clean surfaces in high vacuum. Thus even though the diamond surface is covered by a tenacious film of adsorbed gases, the symmetry of the diamond is projected through the film.

The friction between diamonds in air was first measured by Bowden and Young[23], and Bowden and Tabor[24] later pointed out that their results showed an approximate relation between the friction μ and the load W of the form $\mu \propto W^{-1/3}$. They then suggested that this relation arose because of adhesion processes between the actual areas of true contact, and that this adhesion was responsible for the friction. Recent measurements by Casey and Wilks[25] show, however, that the friction is independent of the load over a wide range of loads, including the range of the earlier experiments. It therefore seems that the mechanism responsible for friction is not one of adhesion, a result which is confirmed by the fact that the friction remains unchanged when the diamond surface is lubricated with light oil.

The observed behaviour of the friction is readily explained by a roughness (or ratchet) type mechanism associated with the jagged nature of a polished diamond surface. That is, the friction force arises from the work required to move the two diamond surfaces against the normal load as they are forced apart when asperities ride over each other. Some of this work will degenerate into heat when the jagged and irregular surfaces come together again, because the return motion will tend to be abrupt and irreversible. The work of separation is proportional to the load, so this type of mechanism leads to a value of the friction which is independent of the load. We believe that this mechanism accounts quite generally for the friction of diamond on diamond in air as discussed in more detail by Casey and Wilks[25]. The adsorbed film acts as a lubricant, which reduces adhesion and the high friction observed in a vacuum, but does not obscure the structure of the surface.

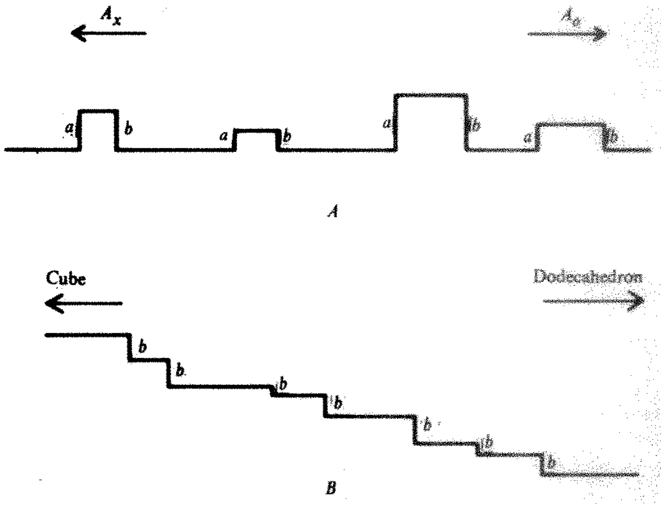

Fig. 3 Schematic diagrams of polished diamond surfaces (see text).

Fig. 4*a* shows the results of recent measurements on the friction of a diamond sliding over a polished cube surface which are similar to those of Seal[7]. The diamond surface had been polished in the usual way and was therefore covered by an array of fine polishing lines or grooves running parallel to a cube axis. Nevertheless, the friction shows a full four-fold symmetry, having the same value in directions parallel and perpendicular to the grooves. We then repolished a surface by abrasion in a hard direction at 45° to a cube axis, until the original polishing lines were replaced by a new set at 45° to the original direction. The friction on this new surface is shown in curve *b*; the friction has quite different values and exhibits only a two-fold symmetry, the lowest values being for directions parallel to the direction of polish.

These results confirm that the topography of a polished surface is determined by mechanical chipping. Just as abrasion proceeds at different rates in different directions, because of the orientation of the cleavage planes, so surfaces prepared by polishing in different directions will have

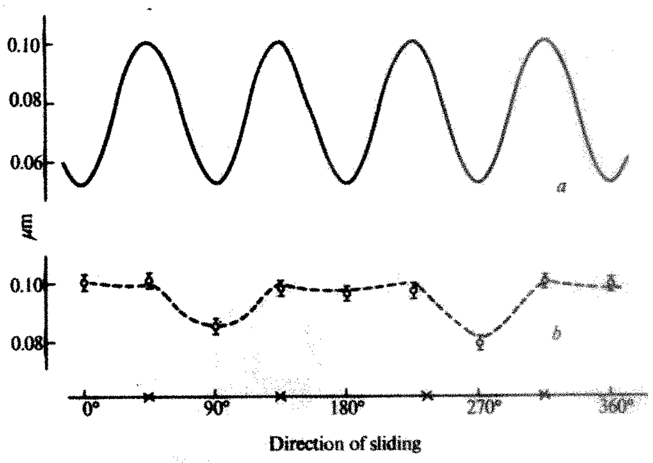

Fig. 4 The friction of a diamond sliding over a polished cube surface as a function of azimuthal angle. *a*, After polishing in the normal soft direction; *b*, after polishing in a hard direction. The symbols × indicate the positions of the cube axes.

different structures. As discussed by Tolkowsky, a surface polished in the usual direction parallel to a cube axis will present a jagged surface with a four-fold symmetry. If, however, the diamond is polished in one of the hard directions, the surface structure will tend to take the form of grooving parallel to the direction of polish, with the two-fold symmetry shown in the friction measurements. We are at present extending our studies to observe the wear effects associated with repeated passes of the stylus over the same area of surface.

The third set of experiments are concerned with observing the surface structure of polished diamonds by using high resolution electron microscopy and carbon replica techniques. The structure turns out to be irregular and on a very small scale; therefore, particular care must be taken to ensure that the micrograph is in focus and free of astigmatism. Fig. 5 shows micrographs prepared under carefully controlled and similar conditions, except that the replica of Fig. 5*a* was taken from a freshly cleaved surface of mica, and those of Fig. 5*b* and *c* from polished surfaces of diamond. The greater contrast in the diamond micrographs indicates that the surface is much rougher than the mica and that the scale of the irregularities is of the order of 50 Å.

The micrographs in Fig. 5*b* and *c* were prepared from the same stone, using the same technique, but Fig. 5*b* was taken from a cube surface polished in the usual way, and Fig. 5*c* from a polished octahedron face. We have also observed similar structures on the cube and octahedron faces of a second diamond, and also a different characteristic structure on the dodecahedron faces. These results confirm the fact, well known to diamond polishers, that the quality of polish depends on the particular face being polished, as follows from the mechanical nature of the polishing process. One expects the surface structure to be delineated, at least to some extent, by cleavage planes. On a cube surface, the traces of these cleavage planes will lie at 45° to the direction of polish, and it is interesting to note that there are features lying in these directions. Further details of our results, together with details of the replication technique, will be published elsewhere.

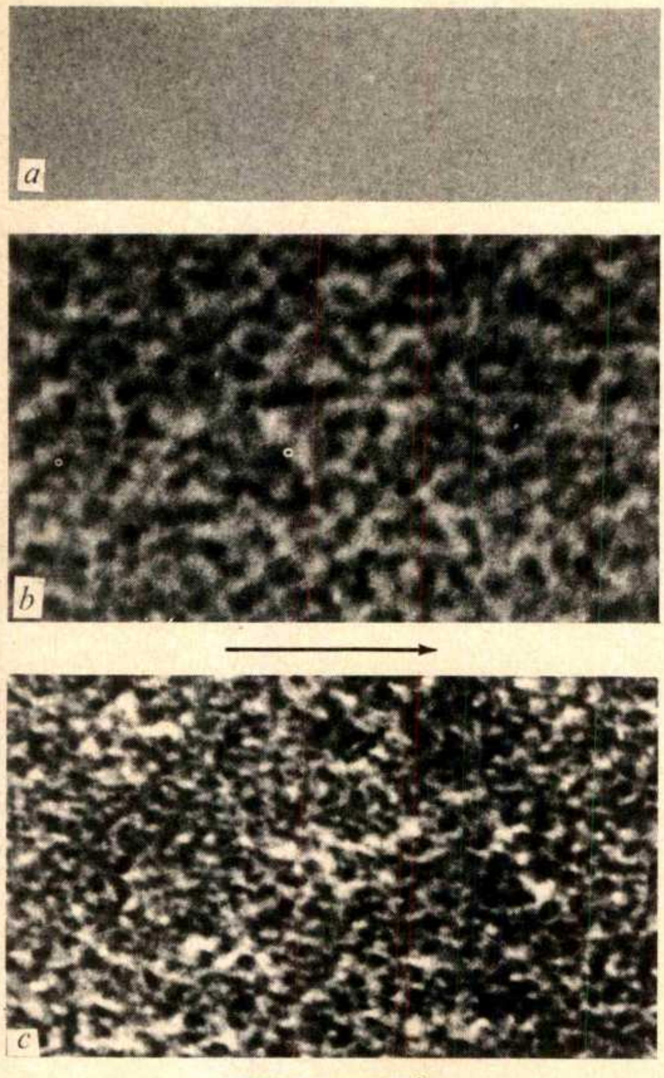

Fig. 5 Micrographs of replicas taken from *a*, cleaved mica; *b*, a polished cube face of diamond; *c*, a polished octahedron face of diamond. The arrows indicate the directions of polish. (×1,000,000).

Diamond is perhaps unique in that an apparently highly polished surface may in fact be mechanically rough on a microscopic scale, a result which is the consequence of its brittleness. We are at present applying these results to the use of diamond as cutting tools for machining metals, as both the rate of wear of the diamond, and the finish produced on the metal, depend sensitively on the surface structure of the tool. For example, the rate of wear of two single-diamond turning tools with identical external geometry, but fabricated so that the diamonds have different crystallographic orientations, show wear rates which are reproducible, and differ by a factor of 7 (ref. 26). The wear in this particular experiment, in which the diamonds turned an aluminium-silicon alloy of the type used to manufacture motor car pistons, was almost certainly controlled by the lie of the fracture planes as discussed above, and these are quite different in the two crystallographic orientations. Further experiments are being continued on both the wear of diamonds and the finish they produce.

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Tectono-eustatic Changes in Sea Level and Seafloor Spreading

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This article describes a model for estimating eustatic changes and evidence for a correlation with seafloor spreading.

EUSTATIC changes in sea level are caused by a change in the form of the Earth's crust or in the total volume of ocean water[1]. The best examples of the latter are the short duration ($\sim 10^5$ yr) reversible changes caused by glacial advance and retreat during the Quaternary although there may also have been an increase in water volume by degassing from the mantle during the Mesozoic and Cainozoic[2,3]. Changes in sea level arising from changes in the form of the ocean basins are revealed in the geological record on continents as longer period ($\sim 10^7$ yr) transgressions and regressions that mark global changes in sea level[4,5], although the magnitude of the change may vary locally. Five principal factors[1,3,6] control sea level; of these, sediment deposition and trench formation may be neglected because the volumes are small or balancd by subduction. The most significant factors are (i) subsidence of the ocean crust, (ii) uplift of mid-ocean ridges and (iii) an increase or decrease in the length of the mid-ocean ridge system. These factors are, of course, controlled by seafloor spreading and their relation to eustatic changes has been subject to speculation[5–13].

Simple Quantitative Model

Here we present a model designed to estimate the magnitude of the eustatic change in sea level that would result from net alterations in the distribution of matter forming the continents and ocean floor, irrespective of the mechanisms involved. We assume a constant volume of water in the oceans and that there is no significant change in the average radius, shape or rotation of the Earth. On a smooth almost spherical Earth covered with the present volume of water, the ocean floor would be about 2,440 m below sea level, which would itself be about 240 m above present sea level[14,15].

Interaction between tectonics and sea level begins only when part of the lithosphere is raised above sea level; then any net vertical movement between the ocean floor and dry area will obviously produce a change in sea level. In particular, any increase in volume of solid crust above the water line must also be accompanied by an absolute lowering of sea level. In the context of plate tectonics, it is most meaningful to retain the distinction between continents and oceans in calculating departures from present continental areas and ocean depths. The global hypsographic curve[16,17] provides a convenient means of analysis (Fig. 1*a*) because constancy of water volume and rock density require that the total area representing crust and water volume must remain constant. To study a systematic series of deviations from the present curve, we varied the continent area (x) and maximum ocean depth (y) independently in 5% increments on either side of the present values, thereby defining forty-eight additional hypsographic curves. The base line for definition of the hypothetical continental areas was taken as the present area of continents at the altitude of present sea level, thus retaining the geophysical concept of a continent which may or may not be covered by water. Further, the profile of the continents was assumed to remain similar to the present profile and the oceanic part was altered by an amount proportional to its depth at all points, thereby preserving important features

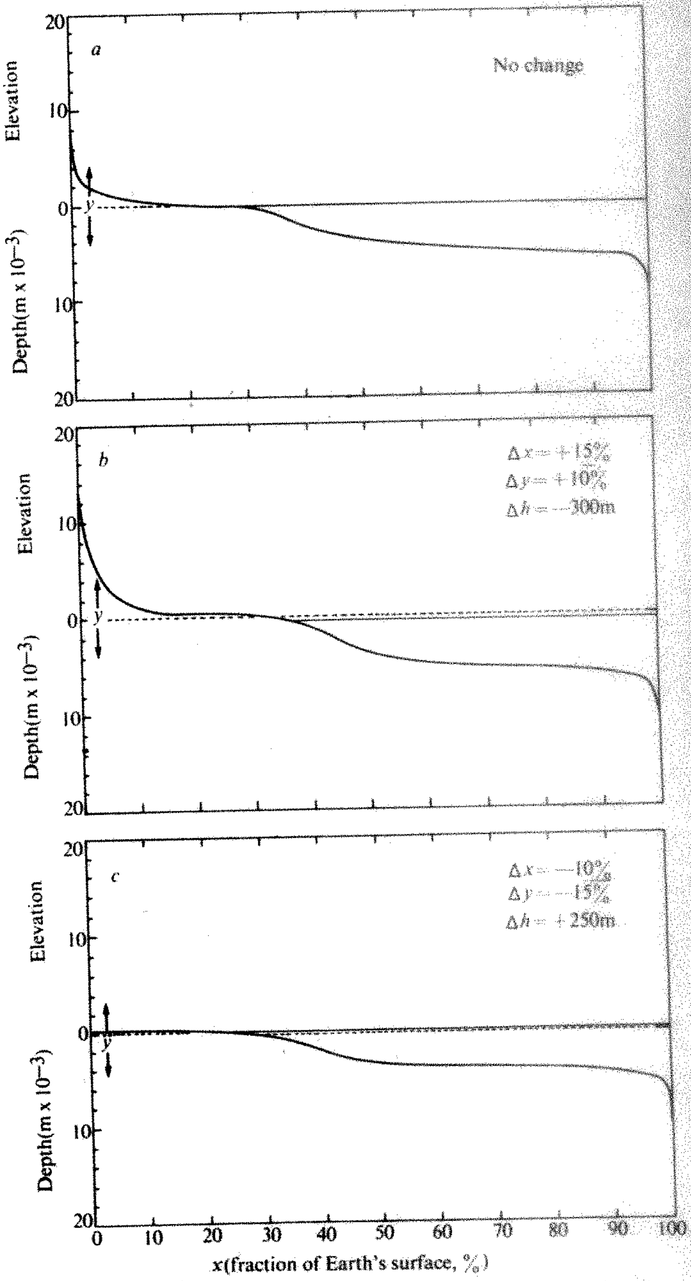

Fig. 1 *a*, The present hypsographic curve for the Earth[16,17]. *b*, Hypothetical hypsographic curve after increasing continental area at present sea level by 15% and increasing ocean depth by 10%. Note increased height of continents, and decrease of sea level by 300 m. *c*, Hypsographic curve resulting from decreasing continental area at present sea level by 10% and decreasing ocean depth by 15%. Although continents are not reduced below present sea level, the eustatic rise in sea level results in total inundation.

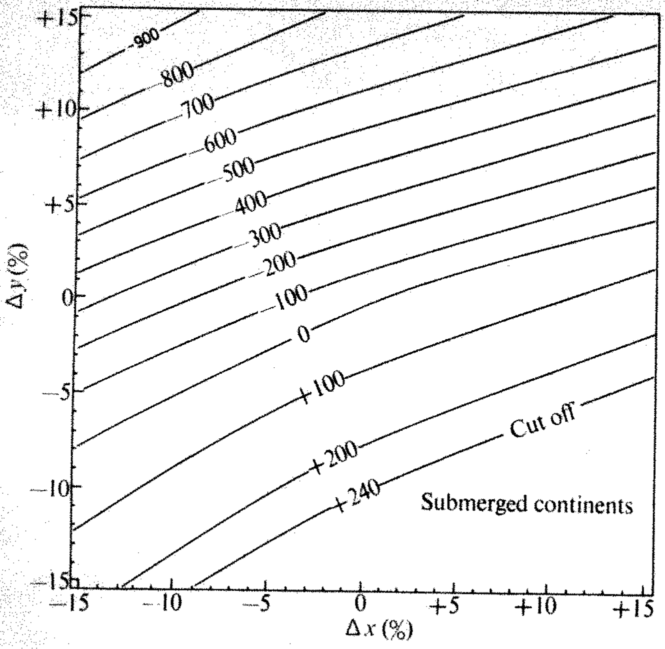

Fig. 2 Eustatic changes of sea level (m) relative to present sea level, contoured in terms of % change in continental area, x, and % change in ocean depth, y. For a rise of 240 m all continents are submerged.

such as continental margins, mid-ocean ridges and satisfying fault distributions observed by Van Andel and Heath[18]; horizontal dimensions were altered in the proportion new oceanic area/old oceanic area.

From comparison with a new hypsographic curve (Fig. 1) we estimated the change in solid volume of the Earth beneath the ocean. This change was added to or subtracted from the volume of the continents above present sea level, and the new profile of the continents calculated using rules of proportionality similar to those used for the oceanic profiles.

Two further boundary conditions are necessary and define the range of variability possible without causing total submergence or extreme altitude. Total submergence of the continents occurs if the postulated increase in Earth volume below the oceans is greater than the total volume of continents above present sea level. Large decreases in solid volume below the ocean, or large decreases in continental area, require excessive increases in continental altitude. Peak continental altitudes greater than twice the present value have been disregarded on isostatic grounds and because uplift is unlikely to exceed erosion by a sufficient amount.

Typical hypsographic curves for two extreme conditions are shown in Fig. 1. For each of the generated curves, the ocean area was drawn in with constant ocean volume and the sea level derived to an accuracy limited by scale to ±50 m. The resulting changes in sea level varied from the maximum rise of 240 m to a maximum fall of 800 to 1,000 m. Errors of about ± 20% were caused by difficulties in constructing the curves and measuring areas.

The model shows that small departures from the present proportion of continental area and ocean depth produce enormous changes in degree of flooding of continental margins (Fig. 2). For example, an extension of continental area results in a lowering of the mean height of continents, and an increase of land near present sea level with a necessary eustatic rise in sea level causing extensive trangression and shallow seas. An increase in continental area of 10% accompanied by a 6% decrease in mean ocean depth would submerge all continents.

Changes and Discontinuities

The oceanic part of a plate steadily subsides as it spreads away from the mid-ocean ridge axis[3,19,20] and the aseismic continental margin undergoes a similar thermal subsidence that declines exponentially with a time constant of about 50×10^6 yr (ref. 21). Transgressions and regressions on young margins may be masked by this effect and are therefore likely to be best observed on margins older than 50 m.y. We have therefore examined the transgressions and regressions shown on Sleep's generalized subsidence curve[21] for the old, Triassic[22] Atlantic margin of the United States for correlations with events in independently derived spreading histories[23,24] for the North Atlantic Ocean (Fig. 3). Our comparison shows a good correlation between the Oligocene regression and deceleration in spreading at 45 m.y. and correlations between earlier eustatic changes and spreading discontinuities.

To check that this correlation does not reflect only the vertical movement of a particular plate, we have examined the Late Cretaceous and Cainozoic spreading histories of the major plates[25] for a global relationship. Global discontinuities in spreading have been postulated[23,25–28]; our comparison (Fig. 4) shows a global deceleration in spreading at about 45 m.y., an acceleration at 10 m.y., and other discontinuities between 55 and 60 m.y. and about 70 m.y. Comparison of the ages of these discontinuities and eustatic changes documented on continental margins (Fig. 4 and (Fig. 5) shows (within the limitations of the data) that the Oligocene–Miocene spreading deceleration is associated with a regression preceded by a minor transgression. The Late Miocene transgression just precedes the 10 m.y. acceleration and a transgressive–regressive phase may correlate with the 60 m.y. discontinuity. We therefore suggest that eustatic changes are connected with contemporaneous global spreading discontinuities and that older transgressions such as the Cainomanian will probably be associated with similar discontinuities.

A relationship between mid-ocean ridge elevation changes and eustatic changes has been suggested[3–13] and the relationship between ridge elevation and spreading rate[29] has been supported by statistical evidence[20] showing that ridges spreading slower than 3 cm yr^{-1} tend to be higher than faster

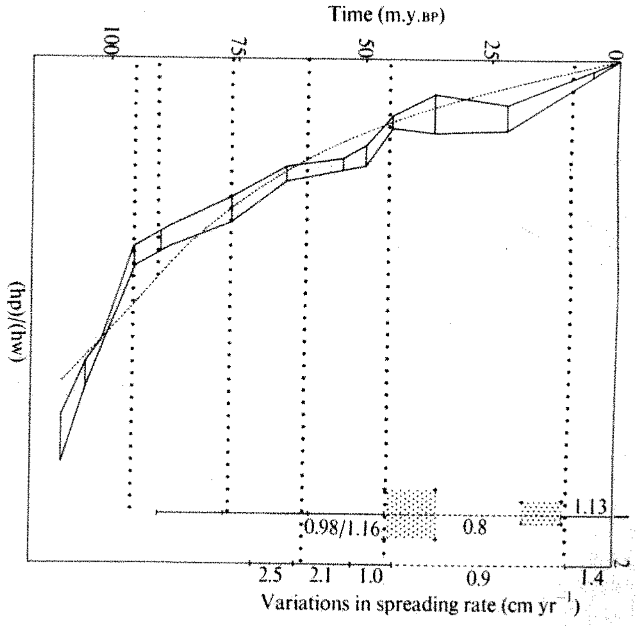

Fig. 3 Subsidence curve (– – –) for the Atlantic continental margin of the USA (from ref. 21). The depth normalized to the base of the Woodbine formation is plotted as a function of absolute age for wells on the East Coast. The error bars are 90% confidence limits. The subsidence curve is a 50 m.y. exponential constraint to fit data at the base of the Woodbine and at the surface. Kinks in the data correspond to eustatic changes. Spreading history of the North Atlantic derived independently is shown in column 1 (ref. 23) and column 2 (ref. 24). . . ., possible correlations between eustatic change and spreading. Shaded area, transition from one spreading mode to another[23].

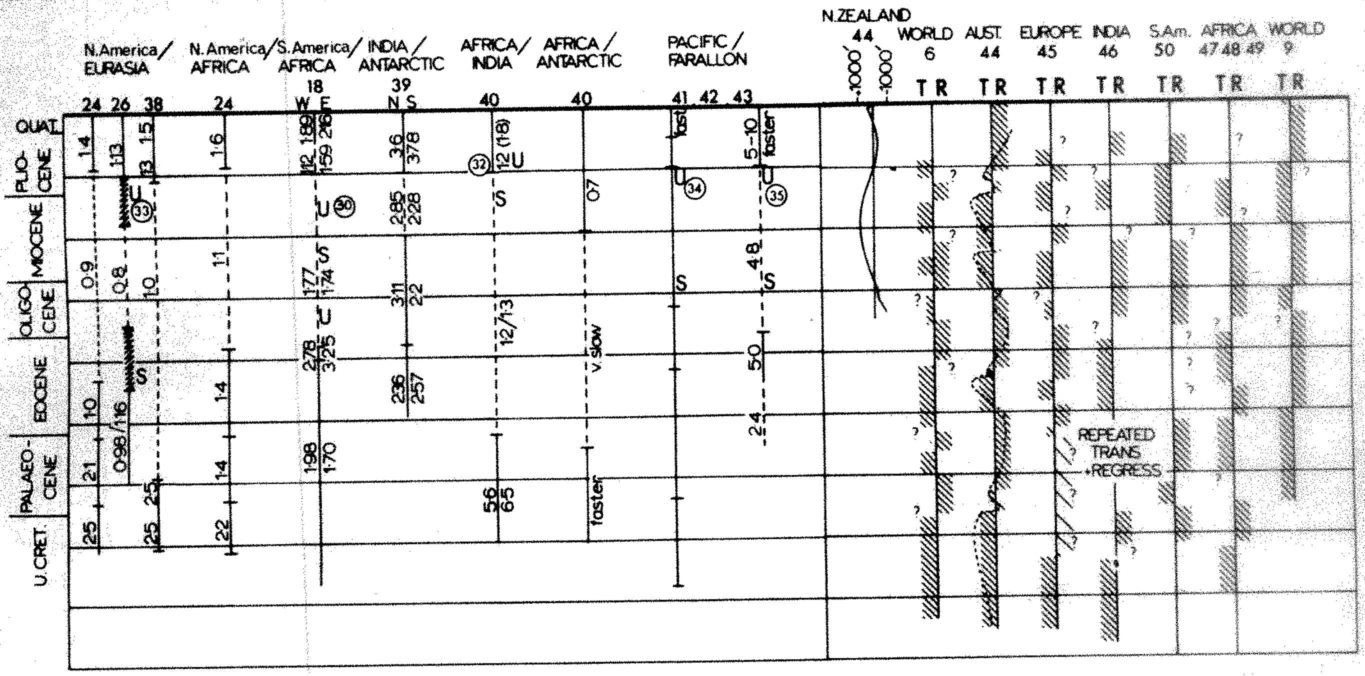

Fig. 4 Correlation between global discontinuities in spreading and global eustatic changes. – – –, General reduction in spreading rate during the Oligocene and Miocene. Note correlation of uplift (U) and subsidence (S) with spreading rate changes and eustatic changes. Eustatic changes are shown as regressive (R) and transgressive phases (T) rather than curves showing interpreted amplitude of change. Circled numbers are references.

ones. Decelerations in spreading should therefore be accompanied by uplift and accelerations by subsidence. But deep-sea drilling data[30] and spreading rates[18] for the southern Mid-Atlantic Ridge show the converse; the post 38 m.y. deceleration was followed by uplift in the Late Oligocene and subsidence in the Early Miocene; the ridge was uplifted to near its present elevation just before the 10 m.y. acceleration. Although these elevation changes correlate well with eustatic changes, the differences between the observed data and empirical model suggest that, at the slow (1.12 to 1.74 cm yr^{-1}) spreading rates on this ridge[18], effects such as phase changes[31] may also contribute to elevation changes. Other deep-sea drilling data[32–35] can also be interpreted to support uplift and subsidence accompanying spreading rate changes. The transgressive–regressive sequence may be due to elevation changes during the adjustment period observed at some discontinuities[26].

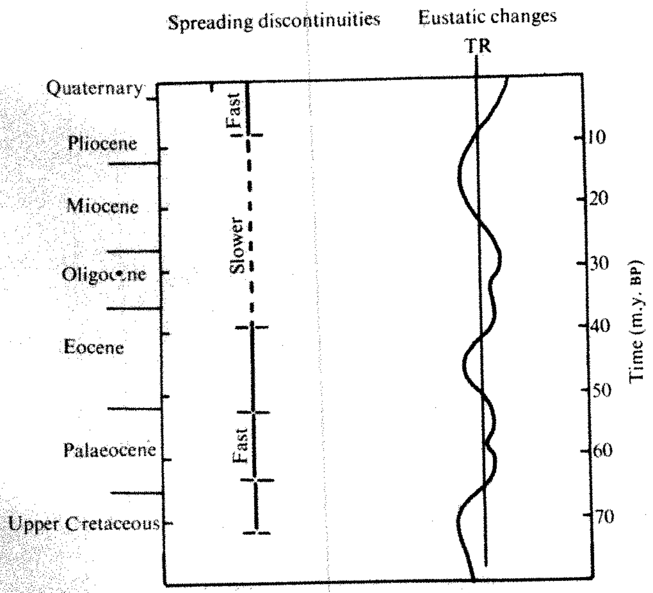

Fig. 5 Correlation between generalized global discontinuities in spreading and generalized global eustatic change (from Fig. 4). The eustatic change is necessarily approximate because of the limitations of the data.

General Implications

The paucity of deep sea drilling data does not permit us to estimate accurately eustatic changes caused by uplift and subsidence. But the southern Mid-Atlantic Ridge was uplifted[30] by between 500 and 1,000 m during the Late Miocene, a change of 25%. If we assume this uplift was not uniformly distributed over the South Atlantic[18] and that uplift on other ridges was not perfectly synchronous, we can reasonably consider a reduction in ocean depth of about 10%. A depth decrease of this order would, provided the continental area remained constant, result in a eustatic rise of about 500 m (Fig. 2) because the change depends on the prior form of the hypsographic curve. If the continental area decreased during uplift, the eustatic change would be rather less but more if the area increased. Estimates of a Middle Miocene transgression of 350 m[36] suggest our calculations are of the right order.

The maximum eustatic rise of 300 m (including 60 m for deglaciation) implies uplift of all marine features now above 300 m. The maximal fall of 800 to 1,000 m indicates that sub-aerial processes may have influenced the morphology of the outer shelf, upper slope and canyons. Such a fall might have exposed sills in semi-enclosed seas resulting in circulation changes and possibly in complete evaporation; a lowering of this order could, for example, have contributed to the drying out of the Mediterranean in the Miocene[37] (see Fig. 2).

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Resonance Raman Spectroscopy of the Photoreceptor-like Pigment of *Halobacterium halobium*

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Use of the resonance Raman technique has shown that the colour of the purple membrane pigment of *H. halobium* arises from an unprotonated Schiff base whose electron density is perturbed by further (electrostatic) interaction with a protein. The chromophore probably consists of a charge transfer complex between retinyllysine and an appropriate side chain of the protein.

RESONANCE Raman spectra have been reported for several molecules of biological interest, including haemoglobin[1,2], cytochrome C[2,3], rubredoxin[4] and several carotenes[5] and retinals[6,7]. The resonant enhancement of certain Raman-active vibrations occurs when molecules are excited by light of a wavelength lying within an electronic transition[8], and the effect provides a structural probe at unusually low solution concentration. Of particular importance to biological systems is the feasibility of *in situ* examination.

One potentially useful area of application for this technique is to photoreceptor pigments. The bathochromic shifts in visual pigments have not yet been satisfactorily explained. The absorption maxima of these pigments, in which there appears to be a Schiff base of 11-*cis* retinal with the ϵ-amino group of a lysine residue, vary from 430 to 562 nm (ref. 9). Solutions of the Schiff base itself, however, absorb near 360 or 440 nm, depending on whether the nitrogen atom is unprotonated or protonated, respectively. Thus another specific interaction occurs between protein and chromophore which perturbs the chromophore absorption band and gives rise to pigment colour[10]. Several theories proposed to explain visual pigment spectra have been reviewed by Abrahamson and Ostroy[11]. The two most important ones are those of: (1) Morton *et al.*[12,13], which assumes that the primary bond is a protonated Schiff base in which the bathochromic shifts are provided by negatively charged groups appropriately positioned adjacent to the polyene chain; (2) the Dartnall formulation[9], which assumes that the primary bond is an unprotonated Schiff base, with secondary shifts caused by an optimally placed pair of charges, producing massive dipoles in the polyene. Little experimental evidence is available with which to test either description.

Because of the anticipated extensive photochemical changes associated with the shining of intense laser radiation on a sample containing rhodopsin, I decided to examine initially the photoreceptor-like pigment of *Halobacterium halobium*. The "purple membrane" fragment containing the pigment offered several advantages for a laser-Raman spectroscopic study. (*a*) The primary structure of the chromophore is retinyllysine, the evidence being much the same as that for rhodopsin[14]. (*b*) Unlike rhodopsin, the pigment does not bleach or undergo photochemical changes on even prolonged exposure to intense (>200 mW) laser light, but simply undergoes a reversible spectral shift from λ_{max}= 558 nm to λ_{max}=570 nm on exposure to red or blue light respectively[14]. (*c*) The membrane is easily handled at room temperature in aqueous suspension.

The purple membrane used in the study described here was the gift of Drs D. Oesterhelt and A. E. Blaurock.

Cells of *Halobacterium halobium* were grown and the purple membrane fragments isolated as previously described[14]. Suspensions of membrane used for Raman spectroscopy had an optical density of 3.0 at λ_{max}.

Raman spectra of purple membrane fragments excited with 4880 Å and 5145 Å radiation are shown in Fig. 1. The most intense vibration in each spectrum occurs at 1,531 cm^{-1} and about fifteen other peaks have also been distinguished. The only significant difference in the two spectra is the relative intensity of the 1,568 cm^{-1} vibration compared with that at 1,531 cm^{-1}. The former appears about four times more intense when excited by 4880 Å radiation than when excited by 5145 Å excitation. This observation is explained below.

Evidence for Resonance Enhancement

Several experiments showed that the observed spectra were resonance-enhanced. (*a*) The protein concentration as measured from the visible spectrum (ϵ_{max}=54,000 l/mol cm)[14] was 5.5×10^{-5} M or two orders of magnitude less than that required to obtain ordinary protein Raman spectra[15]. (*b*) On bleaching the pigment with 0.1 M cetyltrimethylammonium bromide (*p*H 7.9), a procedure which leaves the Schiff base intact but destroys the purple colour[14], no spectrum could be observed. (*c*) An excitation profile (variation of Raman intensity with excitation wavelength) experiment was carried out as follows. Samples of membrane were prepared containing 0.1 M Na_2SO_4 in which the non-resonant enhanced symmetric stretching vibration of the SO_4^{2-} ion at 983 cm^{-1} was used as an internal standard. The ratios of the intensities of the peaks at 1,531 and 1,568 cm^{-1} to the intensity of the standard were determined as a function of excitation wavelength using six lines available from the argon ion laser, all of which lie on the high frequency side of the pigment absorption band. The results are shown in Fig. 2. It is clear that there is substantial deviation from the $1/\lambda^4$ law for intensity of scattered light, as the observed ratios would not vary with wavelength if this relationship were obeyed. The two vibrations studied appear to be enhanced by (vibronic) coupling to different components of the membrane absorption spectrum. The peak at 1,531 cm^{-1} increases in relative intensity as the exciting line increases in wavelength toward the pigment λ_{max}. It is therefore coupled to the main pigment absorption. The 1,568 cm^{-1} band, however, is increased in relative intensity as the wavelength is decreased, and it is therefore not coupled to the visible component of the membrane spectrum but to an absorption located at shorter wavelength than the main band.

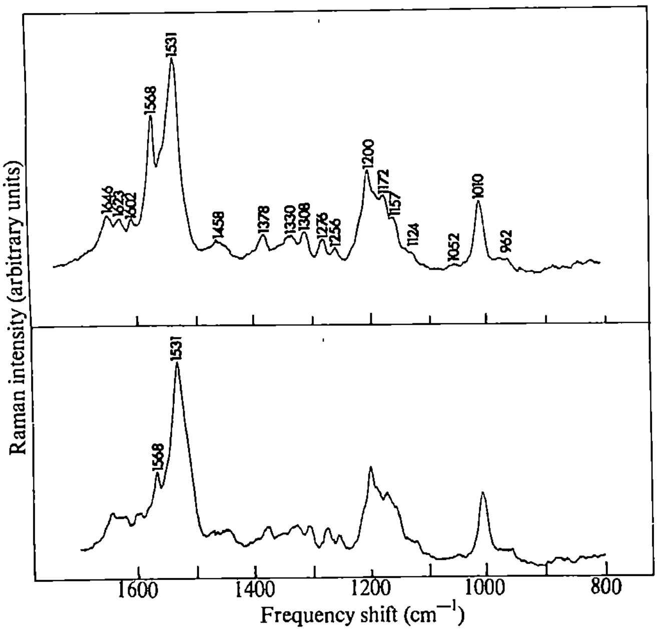

Fig. 1 Raman spectra of an aqueous suspension (5.5×10^{-5} M in the purple protein, *p*H 7.0, unbuffered) of purple membrane fragments obtained with a 'Spex' 1401 Raman Spectrophotometer using: (Top) 100 mW of 4880 Å radiation from an Ar$^+$ laser. Resolution, 7 cm^{-1}; time constant 2 s; scanning rate 25 cm^{-1} min^{-1}; photon counting detection; 1 mm i.d. capillary cell, transverse excitation. (Bottom) 125 mW of 5145 Å radiation from an Ar$^+$ laser. Conditions as above. Polarization measurements indicated that all observed vibrations had depolarization ratios $0\leq\rho\leq0.3$. Spectra of membrane fragments suspended in 4 N NaCl solution are identical with those shown, as are spectra obtained in 0.05 M phosphate buffer (*p*H 7.0).

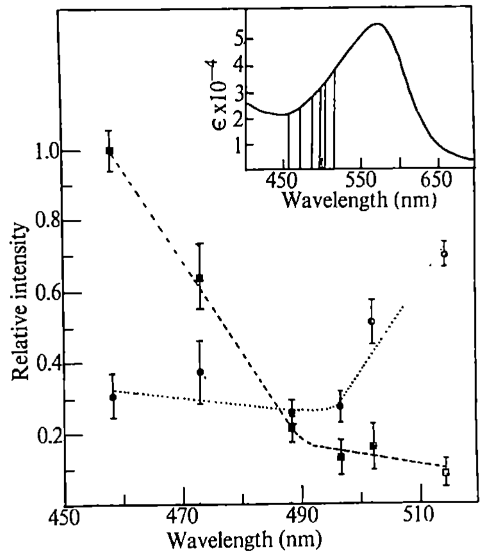

Fig. 2 Excitation profile results. - - -, Ratio of the integrated intensities of 1,568 cm^{-1} vibration compared with 983 cm^{-1} vibration of SO_4^{2-} ion used as internal standard. . . . . , Same ratio comparing the 1,531 cm^{-1} vibration with the standard. ■ and ●, Experimental points, observed at the indicated laser wavelength. Error bars represent standard deviations based on four measurements. Insert, pigment absorption band of purple membrane. Vertical lines indicate positions of laser lines located at 457.9, 472.7, 488.0, 496.5, 501.7 and 514.5 nm. The visible absorption spectrum was recorded on a 'Unicam' SP-800A spectrophotometer using cells of 2 mm path length.

All of the other vibrations (except that attributable to solvent O–H bending at 1,646 cm^{-1}) behave in a fashion similar to the 1,531 cm^{-1} vibration and hence arise from coupling to the 560 nm band of the pigment.

Analysis of Spectral Data

Spectral analysis is simplified by the fact that only chromophore vibrations are observed in the resonance Raman effect[1-3]. Protein vibrations are of insufficient intensity to be seen and therefore do not complicate the spectra. In this work the region 1,500–1,700 cm^{-1} which contains C=C and C=N stretching vibrations will be considered. The region below 1,500 cm^{-1}, which contains C–C stretching and C–H bending modes, will be discussed in a future publication.

Rimai and coworkers have made a detailed Raman spectroscopic study of various retinals[7] and their Schiff bases[8]. They found that those vibrational modes most strongly enhanced are contributed by C=C and C–C stretching in the conjugated chain, which occur near 1,570 and 1,200 cm^{-1}, respectively. In addition, they identified C=O and C=N stretching vibrations in the range 1,600–1,670 cm^{-1}.

In Fig. 3*a* and *c*, the 1,500–1,700 cm^{-1} region of the Raman spectra of unprotonated and protonated retinylhexylamine are shown. The C=N frequencies in this model Schiff base are expected to be similar to those of the pigment Schiff base

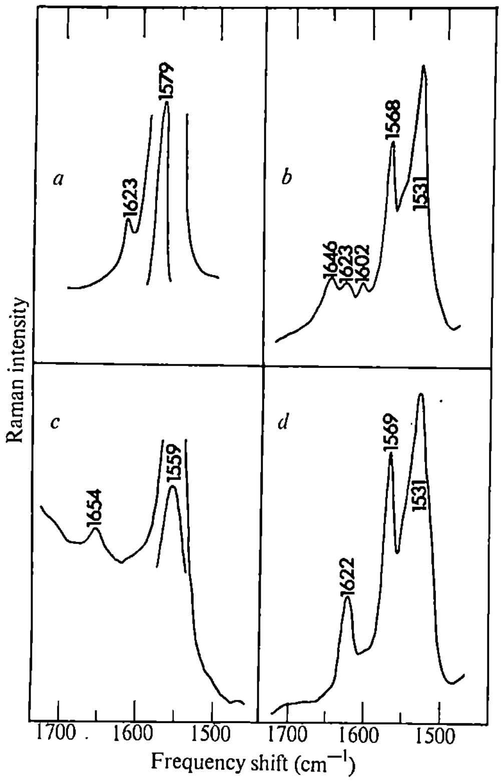

Fig. 3 1,500–1,700 cm^{-1} region of the Raman spectra of *a*, unprotonated retinylhexylamine (10^{-3} M in C_6H_{14} solution, λ_{max}=360 nm); *b*, purple membrane fragments in H_2O suspension (see Fig. 1 (top), caption); *c*, protonated retinylhexylamine (10^{-4} M in acidified EtoH solution, λ_{max}=445 nm); *d*, purple membrane fragments in D_2O suspension (conditions as in Fig. 1 (top) except for solvent). All spectra recorded using 4880 Å radiation. The difference in concentration in *a* and *c* reflects the increased resonant enhancement as the retinylhexylamine absorption band is shifted into the visible region. There is little or no change in the Raman frequencies of this Schiff base as a function of solvent. The best spectra of unprotonated and protonated retinylhexylamine were obtained in the solvent indicated.

and can be used to decide whether the nitrogen of the latter is protonated. In the unprotonated form (Fig. 3*a*), ν (C=N) occurs as a weak band near 1,623 cm^{-1} while ν (C=C) appears strongly at 1,579 cm^{-1}. In protonated retinylhexylamine (Fig. 3*c*) the C=N stretching frequency is broadened and shifted to 1,645 cm^{-1}, while ν (C=C) is shifted to 1,559 cm^{-1}.

In Fig. 3*b*, the 1,500–1,700 cm^{-1} range of the purple membrane spectrum is shown. This region is complicated by the presence of a weak solvent OH bending vibration at 1,646 cm^{-1}, although distinct peaks are seen at 1,531, 1,568, 1,602, and 1,623 cm^{-1}. The peak at 1,568 cm^{-1} is assigned to ν (C=C) of the Schiff base. The excitation profile results described above indicate that this vibration arises from retinyllysine other than that in the pigment. Whether this free Schiff base is significant to the structure of the pigment is not clear. On suspension of the purple membrane in D_2O, the 1,646 cm^{-1} band disappears as expected, while the vibration near 1,623 cm^{-1} becomes more prominent (Fig. 3*d*) than in the H_2O preparation. This band, assigned to C=N stretch of the Schiff base, appears at the same frequency as in unprotonated retinylhexylamine and strongly suggests that the Schiff base in the pigment itself is unprotonated.

The strongest peak in the purple membrane Raman spectrum at 1,531 cm^{-1} has no counterpart in the spectra of the model Schiff bases, all of which have intense C=C stretching vibrations above 1,550 cm^{-1}. The intensity of the 1,531 cm^{-1} band implies strong vibronic coupling to the pigment absorption and it is suggested that, by its magnitude and position, the vibration is still due to retinal C=C stretch. The frequency decrease indicates that the π electron density of the conjugated system has been perturbed and electrons removed from the C=C bonds. Such a process would reduce the C=C stretching force constants and lower ν (C=C).

The above observations indicate that pigment colour arises from an unprotonated Schiff base whose π electron density is perturbed by further (electrostatic) interaction with the protein. Additional evidence for this description was obtained when it was noticed that the purple membrane λ_{max} is highly solvent dependent (C. W. F. McClare, personal communication). Addition of a small amount of chloroform reversibly shifts λ_{max} of the pigment to 500 nm and the main Raman frequency to 1,520 cm^{-1}. The chloroform seems to penetrate to the chromophore and thereby perturbs its electronic arrangement.

The exact geometry of the purple membrane pigment must await a three-dimensional structure determination; however, one model which this study points to is that of a charge transfer complex[16] between retinyllysine and an appropriate side chain of the protein. Such interactions can account for the large shift in λ_{max} (from 360 nm to ~ 560 nm) of the chromophore as well as the perturbation of chromophore vibration frequencies upon complex formation. In addition λ_{max} in such complexes are quite solvent dependent[16], as observed in the present case. That such a mechanism is feasible has been shown by Ishigami *et al.*[17], who observed charge transfer between tryptophan and 9-*cis* retinal in acidified methanol solution (λ_{max} of complex = 520 nm). Furthermore, I have observed charge transfer between indole and *trans* retinal (λ_{max}=625 nm) in the same solvent. It is therefore quite conceivable that an appropriately positioned tryptophan residue on the protein interacts with the π electrons of the Schiff base and produces the purple pigment colour.

A Raman spectroscopic study of rhodopsin and the intermediates present in its low temperature bleaching sequence would yield valuable information as to the nature of the Schiff base-protein interactions that determine pigment colour. The work described here illustrates the feasibility of such studies and the power of the resonance Raman technique in providing a structural probe for the photoreceptor pigments.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Search for Infrared Anomalies associated with Gravitational Events at the Galactic Centre

WEBER[1–4] has reported bursts of gravitational radiation apparently arriving from the direction of our galactic centre (or anti-centre). The resulting mass-loss implied by the estimated energy flux of nearly 100 W cm^{-2} at Earth has caused much theoretical discussion[5–8] with regard to possible source models. This reported high gravitational flux at Earth suggests a search for accompanying electromagnetic bursts from the same direction. Such searches have already been made for microwaves[9,10], X-rays[11,12] and neutrinos[13,14]. All of these searches establish very small upper limits on the ratio of electromagnetic (or neutrino) to gravitational energy flux at Earth; however, radio pulses noncoincident with Weber's pulses have recently been reported[15]. Here we report the results of our search for bursts of infrared radiation from the galactic centre region.

Observations were made from the Cerro Tololo Inter-American Observatory, near La Serena, Chile. Clear nights provided continuous observation periods of up to 8 h. The infrared source coincident with the dynamical centre of the galaxy at RA 17 h 42–43 min and dec −28° 59′ has recently been mapped at 2 μm and 10 μm (refs. 16–18). For observations of this source the 16-inch reflector was used to view a region one arc min in diameter at the centre of the infrared source. Observations covered a period from July 24 to August 8, 1972 (when weather permitted) and totalled 45 h of observing time. Observations were confined to those times when the galactic centre was near the zenith. These times also correspond theoretically to a maximum in the response of an east–west oriented gravitational antenna.

We used a liquid nitrogen cooled PbS detector to view the 2 μm and 4 μm atmospheric windows. Before each observing period, a scan of α Sco was made as a calibration of detector noise and sensitivity. The galactic centre infrared source is first magnitude[16,17,19] in the L band and could be seen with a 2:1 signal-to-noise ratio using a 100 s time-constant. The chart recorder monitor using a 0.1 s time-constant could easily have detected an increase of two orders of magnitude in infrared flux from the galactic centre.

Weber has supplied us with a list of 74 gravitational events covering the period July 23 to August 8, 1972. During our observations, Weber recorded five coincidences between gravitational antennas at the Maryland and Argonne Laboratories. No significant increases in infrared flux above a level of 10^{-14} W cm^{-2} were found within ±1 min of these events. This upper limit on the infrared flux that might be associated with a gravitational pulse is a factor of 10^{16} less than the gravitational flux necessary to excite the coincidences in the gravitational antennas. Since the bandwidth of the 2 μm and 4 μm windows is 2×10^{13} Hz, the energy flux per unit bandwidth in the infrared fluctuated less than 5×10^{-28} W cm^{-2} Hz^{-1} during a gravitational event. This limit is of the order of 10^{26} less than the required gravitational flux per unit bandwidth and is nearly the same as the limit set in the microwave regions[9,10,15].

If the reported high flux gravitational events are confirmed, there are several possible explanations for this absence of observed coincident fluctuations in infrared levels. First, if there is a net polarization of the gravitational radiation, it has been shown that the location of the source of gravitational radiation is not near the galactic centre region[6,7]. Second, dust in the dense region within 1 pc of the galactic centre may absorb most electromagnetic radiation, re-emitting it over comparatively long time periods. Third, there may simply be very little electromagnetic radiation accompanying such gravitational events. Finally, if one assumes no net polarization of the gravitational waves, Weber's data only limit the position of the source to within 4 arc deg of the galactic centre. We have narrowed this search to the small region near the galactic nucleus because the high mass density there makes gravitational radiation more plausible.

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Position of OH471

Carswell and Strittmatter, reporting the high redshift[1] they measured for OH471, do not quote the accurate radio and optical coordinates which were the basis of its identification. These coordinates are:

Radio α(1950)=06 h 42 min 53.3 s ±0.2 s δ(1950)=44° 54′ 33″ ±1″
Optical α(1950)=06 h 42 min 53.1 s ±0.1 s δ(1950)=44° 54′ 31″ ±1″

The radio position was measured by the method described by Adgie *et al.*[2] except that an interferometer spacing of only 1,300 wavelengths was used. The optical position was measured on the prints of the Palomar Sky Survey relative to AGK2 stars: the accuracy achieved was estimated from the distribution of radio-optical position differences obtained in a large number of similar measurements.

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Discontinuous Change in Earth's Spin Rate following Great Solar Storm of August 1972

The question of a link between changes in the Earth's spin rate and the activity of the Sun is of topical interest, and there is good evidence that the changing length of day is influenced by the mean level of solar activity[1,2]. The possibility of a one-to-one correlation between specific events on the Sun and specific changes in the length of day has remained more controversial, however, although there was a suggestion of such an effect associated with the great solar storm of 1959 (refs. 3–5) Specifically, Danjon suggested[3–5] that there was an increase in the length of day when the nucleonic component of solar cosmic rays increased; this was in addition to the usual steady increase in the length of day. Other observers questioned the reality of this effect (for a discussion of the controversy see ref. 6), and because the 1959 solar storm was the greatest recorded since the time of Galileo, there was no immediate hope of an independent test of Danjon's claim. In August 1972, however, an even greater disturbance occurred on the Sun[7–9]. It seemed to us that this might provide the ideal opportunity to resolve the controversy, and we have indeed found a discontinuous change in the length of day, and a change in the rate of change of the length of day (a glitch) immediately after that event. Changes in the length of day, and thus in the spin rate of the Earth, are revealed by regular measurements of Universal Time (UT) carried out at many observatories around the world. For our purpose, we are interested in UT2, the version of Universal Time with the effects of the Chandler Wobble and seasonal variations removed. The difference between Atomic Time (AT) and UT2 shows, on average, a monotonic increase as the Earth's spin slows down and the length of day increases.

In Fig. 1 we plot AT–UT2 for a period of one month on either side of the great solar storm of August 1972. The spot group associated with the flare activity built up from July 29, reaching a maximum size (covering 17° in longitude) on August 4. In Fig. 1, we have marked August 3 (JD 41532), the last day before the series of Forbush decreases which marked the solar activity[7], by an arrow. The expected change in spin rate and length of day is clearly visible; a similar plot using data for the six month period May to October 1972 shows (Fig. 2) that the jump is the largest such event recorded over that period. The change in slope indicated by the data of Fig. 2 is equally significant; this is qualitatively similar to the changes seen in pulsar spin rates during glitches, and we therefore borrow the term from the pulsar literature to describe this terrestrial process. At the time of the flare and sunspot activity, the slope flattens slightly, subsequently trending back towards a slope more typical of the data of the preceding three months over a period of several weeks.

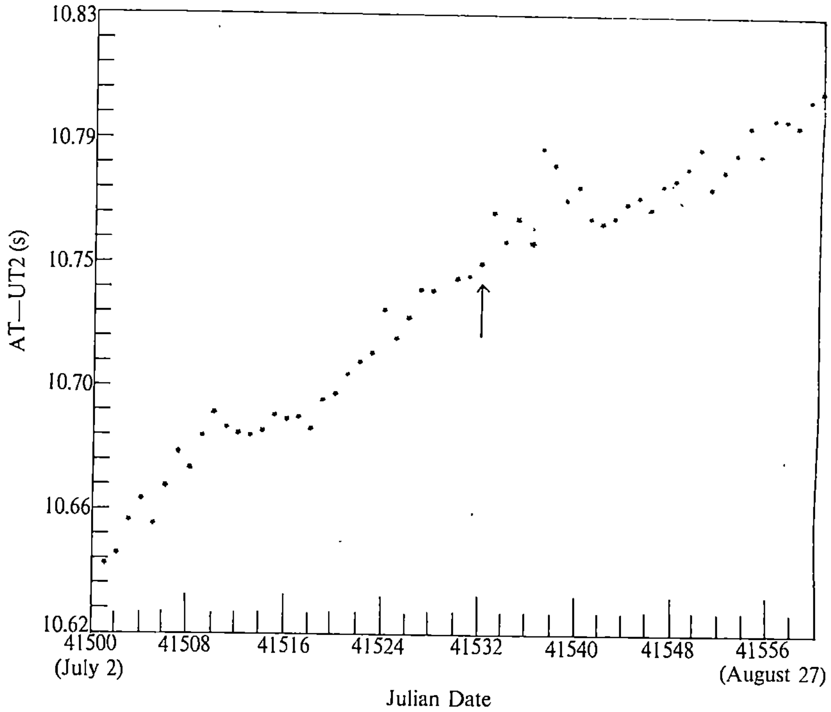

Fig. 1 Change in length of day for a period of one month on either side of date of great solar flare activity of August 1972. Arrow marks August 3, the last day before the flare activity. Large discontinuous change in AT–UT2 occurs on August 8.

These effects are not so dramatic that one would necessarily attribute them to an outside cause on the basis of these data alone, but they take on a greater significance in the light of our prediction, following Danjon, that just such a change should occur soon after a great solar flare. We are confident that the effect is real, and that the glitch was indeed caused by events associated with the solar activity of early August 1972.

It is not difficult to envisage models which explain the delay of 5 days between commencement of the flare activity and the glitch. We will not discuss detailed mechanisms here, except to point out that solar phenomena are known to influence the large scale circulation of the Earth's atmosphere. For example, troughs in the circulation pattern at high latitudes are amplified when the level of solar cosmic rays reaching

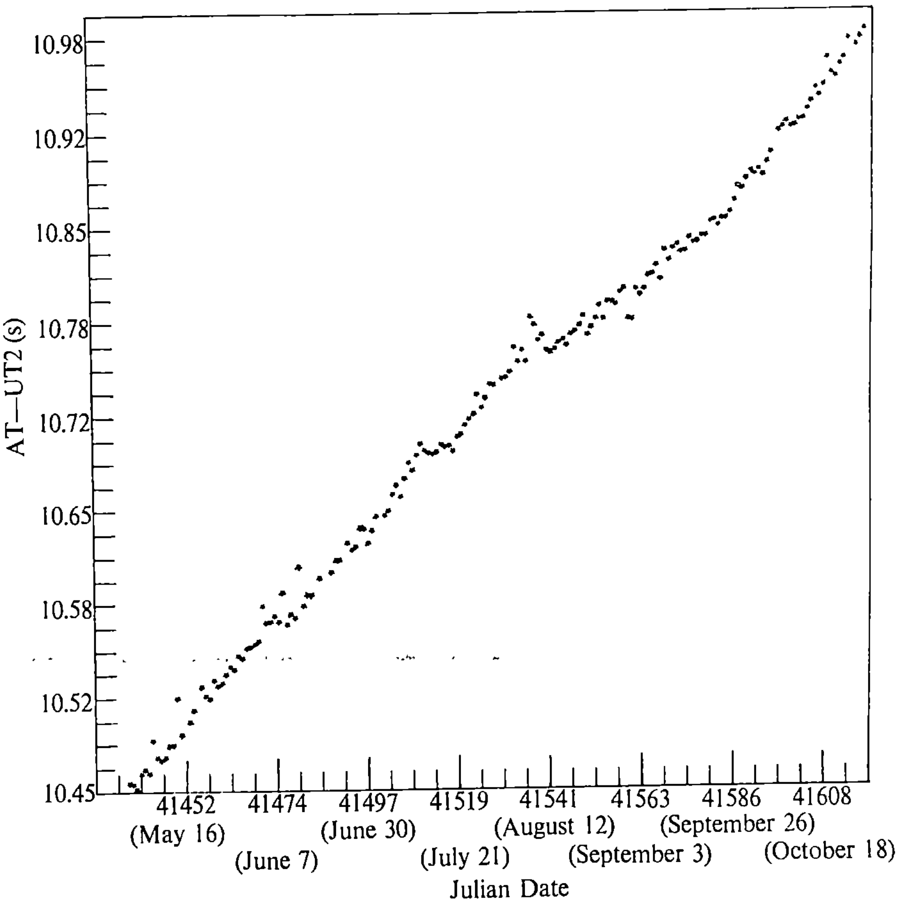

Fig. 2 As Fig. 1, for May to October 1972. The change in slope after the time of the great solar activity, and subsequent return towards the pre-flare slope, emphasize the importance of the event.

the Earth is high[10,11]. Like Schatzman[6], we believe that sudden variations in the length of day may be produced by meteorological phenomena induced by solar activity; in that case, it would be most unreasonable if it did not take a few days for these effects to show themselves in the AT–UT2 measurements.

We will discuss details of such a mechanism and further consequences of this discovery elsewhere. We thank the US Naval Observatory, Washington, for suppling the raw data used in the preparation of Figs. 1 and 2.

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Viscous Remanent Magnetization in Oceanic Basalts

THE magnetic properties of the basalts which form layer 2 of the oceanic lithosphere are important because of their relevance to the hypothesis[1] of seafloor spreading. Most studies of these magnetic properties have been carried out on basalts obtained from dredge hauls taken predominantly from ocean ridge systems and fracture zones. These constitute special areas of the oceanic crust where the sediment cover is negligible. It is of interest to compare the magnetic properties of the dredged basalts with samples recovered from holes drilled through the overlying sediments into the basaltic layer at places distant from ridge axes. Samples obtained from the abandoned Mohole project and, more recently, from the Deep Sea Drilling Project (DSDP) possessed magnetic properties similar to those of dredged basalts[2,3]. Here I describe highly unstable magnetic characteristics found in basalts from DSDP hole 57.

In a study of basalts from DSDP holes in the Atlantic Ocean[3] the natural remanent magnetizations (NRM) were found to be very stable, requiring alternating fields of 200–400 oersted to destroy 50% of the original remanence. The NRM directions and intensities were invariant in storage tests in the Earth's magnetic field lasting several months. High Königsberger ratios, averaging about 11, imply that the remanent magnetizations were much more important than magnetizations isothermally induced by the ambient geomagnetic field. The remanent intensities were considerably lower than those found in dredged basalts (1×10^{-3} gauss compared to about 5×10^{-3} gauss), but were strong enough to account for the observed magnetic anomalies if a thickness of 2.5 km is assumed for a uniformly magnetized layer 2. Palaeolatitudes deduced from the remanent inclinations of extrusive basalts agreed with palaeolatitudes derived from a model reconstruction of the opening of the Atlantic Ocean[4]. The basalt magnetic properties were therefore compatible with the Vine and Matthews hypothesis[1].

Present investigations of DSDP basalts from several holes in the Pacific Ocean have shown that their magnetic properties are similar to those described above and to those of dredge haul samples (W. L., R. Løvlie and N. D. Opdyke, unpublished). But the remanent inclinations were different from those expected at the sites. The inclinations of partially oriented oceanic basalts have been found shallower than anticipated in other studies[2,5–7]. Otherwise the magnetic character of tholeiitic basalts seems to satisfy the demands imposed by interpretations of marine magnetic anomalies in terms of seafloor spreading. But very unstable magnetic behaviour found in hole 57 basalts contrasts markedly with the usual high stability.

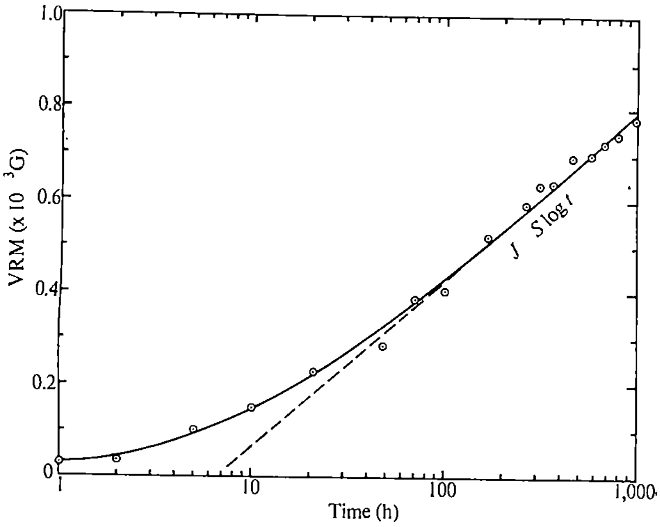

Fig. 1 Acquisition of VRM in a specimen from DSDP hole 57. Core 2–2, depth 60 cm, $S = 0.34 \times 10^{-3}$ gauss.

Hole 57 was drilled at 8°41′ N, 142°32′ E, on the north flank of the Caroline ridge and to the south-east of the Marianas trench. The overlying Late Oligocene sediments were unaltered and the basalt was initially identified as a thick flow (or "lava lake") rather than a sill[8]. Subsequent analysis has given a K/Ar age of 23.5 m.y. for the basalt (I. Ridley *et al.*, unpublished). The basalt is more alkaline than the usual ocean ridge tholeiitic basalt, and is of a type associated with oceanic islands and island chains[6].

The basalt texture was doleritic, by contrast with the fine-grained textures usually found in chilled oceanic basalts. About 20% consisted of clots of large euhedral plagioclase crystals, with a matrix consisting of "ophitic areas containing olivine granules, and patches of quench material rich in oxide skeletal crystals"[9]. From an examination of polished sections with a reflected light microscope, R. Løvlie categorizes the deuteric oxidation state of the basalt as class I, according to the classification scheme of Wilson and Watkins[10]. About half of the skeletal, homogeneous titanomagnetite grains were finer than 10 μm to 200 μm. More usually, titanomagnetite grains in oceanic basalts are less than 5 μm in diameter, and often are too fine for internal features to be resolved by conventional optical microscopy. The Curie temperatures of the specimens averaged 150° C, some 100° C lower than the values found in other DSDP basalts from the Atlantic and Pacific oceans[3]. This indicates that the titanium content of the titanomagnetites was probably higher in the basalts from hole 57, in agreement with observations based on other petrologic evidence.

The remanent magnetizations were measured with a spinner magnetometer. Alternating fields averaging only 40 oersted were capable of destroying 50% of the NRM of each specimen. The magnetic coercivities were so low that adequate demagnetization was impossible. The minimum dispersion of the remanent inclinations occurred after demagnetization in a peak field of 150 oersted. The mean inclination was then 16°±4° (standard error), which was not significantly different from the axial dipole field inclination (17°) at the latitude of the site.

After demagnetization in fields of only 150 to 200 oersted the magnetization was observed to change rapidly while it was being measured, apparently because of the decay of very soft viscous remanent magnetization (VRM) components. Similar viscous magnetic behaviour has been reported in DSDP basalts from the southern Caribbean[6].

Eight of the unstable specimens were demagnetized in peak alternating fields of 300 oersted, and placed in a field-free space for 2 days. A field of 0.53 oersted was then applied in a known direction relative to each specimen. The remanences were measured at intervals, spaced approximately logarithmically, for 1,000 h (6 weeks). In each specimen the remanent magnetization slowly increased in intensity and its direction approached that of the applied field. The acquisition of this VRM followed the same pattern in each case (Fig. 1). Initially the acquired VRM interacted with the remaining part of the NRM, but after 50 to 100 h the growing VRM dominated the process and the magnetization (J) increased logarithmically with time (t) as $J = S \log t$, which is characteristic of VRM[11–14].

The magnetic viscosity coefficient, S, was measured for each specimen from the slope of the linear portion of each semilogarithmic graph. These values for S gave the intensities for the VRM that could have been acquired since the time of the last well-established reversal of the geomagnetic field, 0.69 m.y. ago (Table 1).

The VRMs acquired in 1,000 h were next demagnetized

Table 1 Measured Values of the Magnetic Viscosity Coefficient (S), the VRM Acquired in 1,000 h in a 0.53 Oersted Field ($VRM_{1,000}$), and the Alternating Field Required to Erase 90% of that VRM ($H_{1,000}$)

Specimen No.	S	Magnetization ($\times 10^{-4}$ gauss)			Demagnetizing field (oersted)		
		$VRM_{1,000}$	$VRM_{0\cdot69M}$	NRM	$H_{1,000}$	$H_{0\cdot69M}$	H_{NRM}
57–2–1–83A	3.2	8.3	30	42	59	190	107
57–2–1–113A	3.9	9.2	36	24	32	105	125
57–2–1–148A	3.2	7.9	30	69	40	130	95
57–2–1–148C	2.8	7.5	26	32	38	125	150
57–2–2–48A	3.0	7.3	28	69	109	355	100
57–2–2–60A	3.5	7.9	32	33	137	450	130
57–2–2–149A	3.9	9.3	36	28	42	135	130
57–3–1–12A	3.5	8.7	32	26	53	170	120

Computed values of the VRM that could be acquired in 0.69 m.y. ($VRM_{0\cdot69M}$) and the field to erase that remanence ($H_{0\cdot69M}$) are shown in comparison to the NRM intensity and its 90% erasure field (H_{NRM}).

progressively in alternating magnetic fields. From the demagnetization curves the field ($H_{1,000}$) required to destroy 90% of the VRM was determined. The field needed to destroy a VRM is proportional to the logarithm of the acquisition time[15,16]. Therefore, it is possible to deduce the field ($H_{0.69M}$) that would be required to destroy a VRM acquired since the beginning of the Brunhes normal polarity epoch from $H_{0.69M}=H_{1,000}\times\log\,(0.69\text{ m.y.})/\log\,(1,000\text{ h})=H_{1,000}\times3.26$ (see Table 1).

The data indicate that both the intensity and the stability of the natural remanent magnetization of the hole 57 basalt can be accounted for by a magnetization which is almost entirely viscous, acquired since the beginning of the Brunhes epoch. Viscous remanences such as these, requiring alternating fields of one or two hundred oersted for erasure, have previously been reported in other titanomagnetite-bearing igneous rocks[16]. It is interesting that in spite of the viscous nature of their remanences the samples had Königsberger ratios ranging from 2 to 7, contradicting the suggestion that ratios greater than 1 in igneous rocks imply single domain thermoremanent magnetization[17]. The presence of an appreciable coarse-grained titanomagnetite fraction suggests that the remanence is a multidomain type VRM.

In the Brunhes epoch there are several postulated short reversals, some of which may only represent excursions of the geomagnetic field rather than true reversals[18-22]. Some of these short events may be different recordings of the same event. Their durations are thought to be only 2,000–6,000 yr. The effect on the intensity of VRM that may have been acquired during the predominantly normal epoch would be to reduce it by only a few per cent, and their possible existence does not alter drastically the computed VRM for that period of time.

Available information for oceanic ridge systems that have associated linear magnetic anomaly patterns indicates that the underlying basalts possess stable magnetic properties that account for these anomalies. Most submarine basalt magnetic studies have been carried out on fine-grained ocean ridge tholeiites obtained from dredge hauls or deep sea drilling. The unstable character of the basalt from hole 57 is quite different from that found in ocean ridge basalts. The basalt is also different in that it is probably an oceanic island type (I. Ridley *et al.*, unpublished), more differentiated than ocean ridge tholeiites. The iron oxides have a slightly higher titanium content than usual and are coarser in grain size.

The basalts that form oceanic islands, island chains, seamounts and ocean bottom lava flows arising from volcanic activity distant from ridge systems are genetically different from oceanic ridge tholeiites. If the hole 57 basalt is typical of this group then unstable magnetic behaviour may be more common in oceanic basalts than previously supposed. Unstable and very low coercivity NRM has been observed in other DSDP basalts from the Eastern Atlantic[5] and VRM has been detected in DSDP basalts from the Caribbean Sea[6]. Where such basalts form the oceanic basement magnetic anomalies would only reflect topographic influence and local variations in magnetization contrast. A correlatable linear magnetic anomaly pattern would be absent.

Several explanations have been offered for the existence of regions of uncorrelatable magnetic anomalies, or quiet zones[23], and it may be that no unique explanation will serve for all these areas. None of the explanations is based upon measured rock magnetic properties. The results of this study of the magnetic instability in hole 57 basalts suggest another possible explanation which may be applicable to some quiet zones. The lack of significant anomalies in these quiet zones may be due to viscous remanent magnetization of the underlying crust.

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Comments on "Is the African Plate Stationary?"

Burke and Wilson[1] suggested that the African plate became stationary some 25 m.y. ago after having moved northeast from the Mid-Atlantic ridge since the opening of the South Atlantic. Like Rhodes[2] they believe that the continent drifted over hot spots or convective plumes in the mantle and that Walvis Ridge and the Discovery and Meteor seamount chains represent trails of hot spots marking the relative motion of Africa.

The west coast of Southern Africa is characterized by linear belts of plutonic and volcanic complexes; if these rocks were generated over plumes and the African plate has moved laterally over them they should appear progressively younger in the same direction, from east to west.

The hypothesis may therefore be tested by examining the isotopic age data for the lineaments. This has been done by Marsh[3] for the Upper Mesozoic alkaline igneous complexes of Angola and South West Africa (Fig. 1), demonstrating that no sensible pattern in the variation of ages exists. The available data range from 123 m.y. to 164 m.y. (refs. 4, 5) and do not support the mantle plume theory. Similar results were obtained for two E–W lines of alkaline complexes in Brazil[3] where the ages range from 132–80 m.y. and 80–51 m.y. respectively[6], showing no directional pattern where, according to the hot spot hypothesis, younger ages should be expected from W to E.

Instead, Marsh[3] correlated the lineaments of alkaline igneous activity with transform faults now active, offsetting the Mid-Atlantic ridge, and concluded that the Upper Mesozoic complexes reflect the initial rifting of the Africa–South America plate some 130 m.y. ago.

The oldest lineament of igneous activity along the west coast of southern Africa consists of the Kuboos–Tatasberg–Bremen–Garub line (Fig. 1) and is lower Palaeozoic. The Kuboos Granite was dated at 550±20 m.y. (ref. 7) and provisional data indicate an age of 560 m.y. for the Bremen complex further northeast. A pyroclastic sill associated with the carbonate-bearing diatremes at Garub yielded 500±5 m.y., but it is uncertain whether this age represents the time of extrusion or a later regional thermal event (A. K., H. Allsop and H. Welke, unpublished). Nevertheless, no systematic change in age following the 250 km long lineament is apparent.

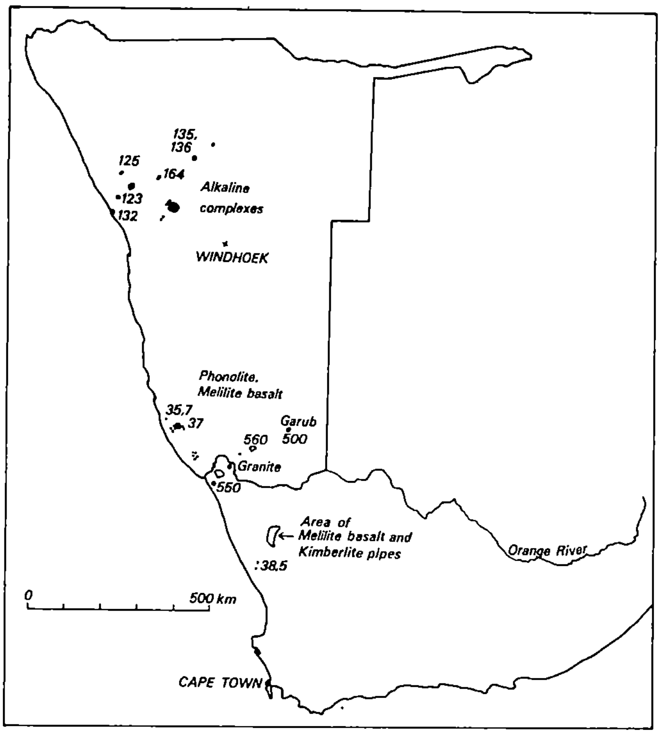

Fig. 1 The early Palaeozoic, Upper Mesozoic and Tertiary lines of volcanic complexes along the west coast of southern Africa. Numbers are radiometric ages in m.y.

Burke and Wilson[1] maintain that such surface structures can only be related to those of the underlying asthenosphere if a plate is temporarily at rest. The alkaline complexes of South West Africa clearly follow a NE trend which reflects the regional structures of the underlying Palaeozoic Damara Orogen, yet the African plate had already begun to drift at the time of alkaline igneous activity[8,9].

During the Oligocene the coastal areas of southern South West Africa and Namaqualand were affected by volcanic activity over a length of some 450 km (Fig. 1). The lines of phonolite and melilite basalt plugs are clearly oriented in a NNW direction and follow structural trends of Late Precambrian origin. Their ages range between 35.7 and 38.5 m.y. (J. A. S. Adams, personal communication) when, according to Burke and Wilson, the African plate was still in motion.

Clearly, lines of volcanic activity in southern Africa are related to older structures in the crust without the African plate necessarily being at rest. I believe that none of the lines mentioned here represents trails of hot spots and I am not aware of any line of volcanoes in Africa that shows a consistent age pattern in any direction.

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Removal of Xenon and Radon from Contaminated Atmospheres with Dioxygenyl Hexafluoroantimonate, O_2SbF_6

RADIOACTIVE noble gases, such as ^{133}Xe, ^{135}Xe, ^{85}Kr, and ^{88}Kr, are formed in uranium fission and are released to the atmosphere by nuclear power plants and fuel reprocessing plants. Studies of the US Public Health Service have shown that a modern boiling-water reactor, for example, releases about 20 Ci of ^{85}Kr and 13,000 Ci of ^{133}Xe per year under normal operating conditions[1,2]. Under adverse operating conditions, the release rates may be much higher[1]. Of the physical methods for reducing these emissions[3–7], cryogenic distillation and charcoal adsorption are being developed for large-scale use.

Soon after the first noble gas compounds had been discovered[8–12], Pomeroy[13] suggested that chemical methods might be useful for trapping krypton and xenon isotopes. Slivnik[14] has studied reactions of krypton-xenon mixtures with fluorine for waste-gas treatment and has demonstrated that xenon can be separated from krypton by such reactions. Fluorine is not, however, a convenient reagent for this purpose, as it must be heated with the process gas, and the excess fluorine must be removed afterwards. I have shown that radon, the heaviest noble gas, can be collected by oxidation with liquid bromine trifluoride and solid halogen fluoride-metal fluoride complexes, such as ClF_2SbF_6, BrF_2SbF_6, BrF_2BiF_6, and $IF_4(SbF_6)_3$ (refs. 15 and 16). I report here further experiments with a dioxygenyl salt, O_2SbF_6, which reacts with radon and xenon at 25° C and which appears very promising as a reagent for removing both of these gases from contaminated atmospheres.

Dioxygenyl hexafluoroantimonate was prepared in 0.5–10 g amounts by photochemical reaction of oxygen, fluorine, and antimony pentafluoride[17]. Samples (0.5 g) of the compound, a white, crystalline powder, were exposed to samples of radon, xenon and krypton in 300 ml. 'Pyrex' bulbs on a metal vacuum line. Tracer ^{222}Rn (8.1 mCi) reacted with the powder immediately at 23–25° C, forming a nonvolatile radon compound. Xenon at 10–200 mm pressure also reacted immediately, forming a pale-yellow, translucent xenon compound. Oxygen was shown to be liberated in the xenon reaction by the fact that the residual gas was not completely condensible at −195° C. No reaction of krypton at 485–700 mm pressure was observed from 23° to 150° C.

Flow experiments were carried out with O_2SbF_6 powder and samples of air (0.33–0.76 l. at STP) which had been artificially contaminated with ^{222}Rn and ^{133}Xe. In each experiment, a rare gas/air mixture was passed through a glass U-tube packed with the powder, then through a trap cooled with liquid nitrogen to condense any unreacted radioisotope. The distribution of the radioisotope was afterwards determined by measuring the γ-emission of the U-tube and the cold trap. (The distribution of ^{133}Xe was determined immediately; the distribution of ^{222}Rn was determined after 3 hours, when ^{222}Rn and its γ-emitting daughters ^{214}Pb and ^{214}Bi were known to be in radioactive equilibrium.) In three experiments with the radon isotope and with a bed of powder 5.0 cm long and 6.3 mm in diameter, all of the radon was absorbed. In 5 experiments with the xenon isotope and with a bed of powder 6.5 cm long and 5.5 mm in diameter, 67–100% of the xenon was absorbed (Table 1).

Table 1 Removal of Radon and Xenon from Air with O_2SbF_6 at 23–25° C

Radioisotope	Conc. in air (mCi $l.^{-1}$)	Average flow rate (ml. min^{-1})	Amount of radioisotope removed (%)
^{222}Rn	13	12	100
,,	15	15	100
,,	24	12	100
^{133}Xe	9.8	15	67*
,,	7.9	13	100
,,	2.0	14	100
,,	3.2	13	98
,,	4.3	14	100

* Flow rate poorly controlled at start of experiment.

Raman spectral studies have shown that the xenon product is $XeF^+Sb_2F_{11}^-$, a 1 : 2 xenon difluoride-antimony pentafluoride complex[18–20]. When xenon is added gradually to O_2SbF_6, a new band (characteristic of the xenon-fluorine stretching vibration of XeF^+ cation[15]) appears in the spectrum at 618 cm^{-1}. Shifts in the SbF_6^- vibration frequencies also occur. The final spectrum contains prominent bands at 450, 618, 655, and 686 cm^{-1}.

Mass spectrometric analyses of residual gases in experiments with krypton-xenon mixtures have shown that two molecules of oxygen are released for each atom of xenon absorbed as follows

$$Xe^{(g)} + 2O_2^+SbF_6^{-(s)} \rightarrow XeF^+Sb_2F_{11}^{-(s)} + 2O_2^{(g)}$$

No spectral or analytical data have been obtained for the trace amounts of radon product (no stable isotopes of radon are known, and the ^{222}Rn product is intensely radioactive), but radon probably forms an analogous 1:2 radon difluoride-antimony pentafluoride complex as follows

$$Rn^{(g)} + 2O_2^+SbF_6^{-(s)} \rightarrow RnF^+Sb_2F_{11}^{-(s)} + 2O_2^{(g)}$$

Mixtures of approximately three parts O_2SbF_6 and one part antimony pentafluoride by volume (grey powders, prepared in a dry box) react with radon but not with xenon. Dilution with antimony pentafluoride therefore lowers the free energy of O_2SbF_6 below the threshold for zenon oxidation but not below that for radon oxidation.

Equimolar mixtures of krypton and xenon were partly separated on passage through U-tubes packed with O_2SbF_6. The mixtures were passed through beds of the compound 5.8–6.0 cm long and 5.5 mm in diameter at 23–25° C and at total pressures of 20–160 mm. Gases emerging from the beds were collected and analysed by mass spectrometry. The first samples had the following range of composition: 45.5–49.6% O_2, 12.7–16.2% Xe, and 37.7–38.3% Kr. The percentage of oxygen fell and that of krypton and xenon rose as the O_2SbF_6 was depleted. The colour change of the powder, from white to yellow, could be used to indicate the amount depleted, as there was a sharp interface between coloured zones. Each time that gas was admitted, a blue-green fluorescence was noted in the O_2SbF_6. The source of this emission is not known at present, but it is probable that an excited xenon, oxygen, or ozone species is formed during the reaction.

More complete separation of krypton and xenon was achieved by shaking equimolar mixtures of the gases with O_2SbF_6 powder in 'Pyrex' bulbs containing stirring vanes. The mixtures were shaken intermittently, by hand, at room temperature with excess amounts of the powder over periods of 3 to 24 h. Residual gases were then analysed by mass spectrometry, and the final gas mixtures were found to contain less than 2% xenon.

Because it has negligible vapour pressure at 25° C, and because oxygen is the gaseous product, the dioxygenyl compound appears to be a very "clean" reagent for air-purification purposes. It is less corrosive than such complex fluorides as ClF_2SbF_6, BrF_2SbF_6, and BrF_2BiF_6, and may be stored in glass or fluorinated plastic containers. Very little decomposition has been noted, for example, in samples that have been stored for 6–9 months in 'Pyrex' bulbs and 'Kel-F' test tubes. Some corrosion of 'Kel-F' has been observed, however, by products formed in the reaction with xenon—probably excited xenon, oxygen, or ozone species. The compound is decomposed by moisture; in treatment of wet gases, it must therefore be used in conjunction with a desiccant, such as silica gel or a molecular sieve.

The dioxygenyl compound can probably be used for such purposes as purifying radon-contaminated atmospheres in uranium mines; removing xenon isotopes from reactor and reprocessing plant off-gases; separating xenon from lighter noble gases; and analysing xenon and radon isotopes in air. In one of the methods which is being developed to reduce emissions from boiling-water reactors, large beds of charcoal are used to "hold up" radioisotopes[2,3]. Waste gases are passed through the beds at ambient temperature, and the noble gases are adsorbed and retained long enough for short-lived isotope to decay. (Xenon isotopes may be held for 20 days and krypton isotopes for about 1 day.) Small beds of O_2SbF_6 can probably be substituted for the large (100 to 200 ton) beds of charcoal to remove xenon isotopes from the waste gases completely.

Further research may disclose oxidants that can be used to collect krypton as well as radon and xenon. Krypton forms a simple fluoride, KrF_2, which is stable only at low temperatures, and a complex fluoride, $KrF_2.2SbF_5$, which is stable at 25° C (refs. 21, 22). (The latter is probably an ionic compound, $KrF^+Sb_2F_{11}^-$, analogous to $XeF^+Sb_2F_{11}^-$.) By substituting cations of very great oxidizing power for the O_2^- cation in O_2SbF_6, it may be possible to prepare reagents that combine spontaneously with krypton to form this stable compound.

I thank J. G. Malm for information concerning the preparation of dioxygenyl hexafluoroantimonate and A. Engelkemeir for mass spectrometric analysis of gas mixtures. This work was performed under the auspices of the US Atomic Energy Commission.

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Inverse Mass Dependence in Liquid Diffusion of Heavy Ions

AFTER the failure of early attempts to find isotope effects in liquid diffusion it was assumed[1] that these effects would be destroyed by two mutually antagonistic mechanisms. Firstly, a first approximation gas model resulted in higher speed for the lighter isotopes; secondly, however, in liquids the lighter isotopes would meet with an increased backscatter and would have less persistence than the heavier isotopes. Thus the preference of the mass independent Stokes-Einstein equation appeared justified for a first approach to liquid diffusion coefficients. The slower diffusion of the lighter alkali and alkaline earth ions was attributed wholly to the larger diameters caused by hydration and not to mass effects. The contribution of hydration will be indeed substantial for the lighter ions such as Li and Na but this influence has never been proved quantitatively especially for the heavier ions such as Sr and Ba.

The hydrodynamic model has been questioned by Lindemann[2]. He suggested a kinetic model and explained the slow motion and the high solvent transfer of the Li ion by momentum transfer.

The kinetic approach of Arnold[3], however, did not take into account the persistence correction. Without this correction by Jeans[4] the Stefan-Maxwell equation could not explain the weak composition dependence of the diffusion coefficients in gas mixtures. In the case of a few heavy molecules diffusing in an excess of light molecules the persistence correction results in a substantial increase in the diffusion coefficient against the uncorrected value.

Because of the higher collision number in liquids the effect of persistence should play a still more important role in liquids than in gases. Recently[5] liquid diffusion cascades have been used in the search for isotope effects in liquid diffusion. A normal gas-like isotope effect was found for the Li isotopes in polyethylene glycol (PEG) solution but the inverse effect occurred for the U isotopes. Only one experiment has shown the same mass dependence: Cini-Castagnoli *et al.*[6,7] investigated the diffusion of argon in liquid nitrogen and found slower diffusion for ^{37}A than for ^{40}A. The difference was up to 10% but it was not emphasized and was apparently considered to be within the range of experimental error.

Here I report further confirmation of this inverse mass dependence for diffusion of uranyl ions in aqueous solution and in agar gel. A prismatic cascade[5] 60 cm high, with a cross section 10 by 60 cm at the top, containing 59 barriers of nylon gauze, was filled with distilled water. Uranyl nitrate solution with density 1.194 was introduced at the top. A layer of glass powder on the top barrier prevented convection of this concentrated solution. After 21 days a sample was drawn from the bottom which showed a depletion of ^{235}U against the blank. The result is given in Table 1. The simple process factor ε, which may be deduced from these results, has the same order of magnitude as previously[5]. Hydration of the uranyl ion does not suppress appreciably the isotope effect.

It was possible to improve the efficiency of the barriers by using cylinders of sintered polystyrene beads impregnated with agar gel or solid agar cylinders supported by stainless steel gauze. The diffusion proceeded in radial direction towards a central channel. Details of this method will be published elsewhere. Again a depletion of ^{235}U occurred as shown in Table 1.

Table 1 Depletion of ^{235}U in Solution after Passage through Barriers

System	Stage No.	Cascades		Diffusion time	^{235}U/^{238}U
		Type	Material of barrier		
Uranyl nitrate in water	59	Prismatic	Nylon gauze	21 days	Sample: 0.007191 ± 0.000013* Blank: 0.007418 ± 0.000015*
Uranyl nitrate in agar gel	2	Cylinders 47 mm diameter	Agar gel in polystyrene matrix	3 days per stage	Sample: 0.007132 ± 0.000019* Blank: 0.007369 ± 0.000029*
Uranyl nitrate in agar gel	1	Cylinder 125 mm diameter	Agar gel	4 days	Sample: 0.007208 ± 0.000022 Blank: 0.007285 ± 0.000018

* Arithmetical mean from 2 runs.

I conclude that the mechanism of persistence and backscatter plays an even more prominent role in the diffusion of heavy solutes in light solvents than was assumed by Hevesy and Paneth[1]. This mechanism not only compensates but overcompensates the features of the gas model and results in inverse mass dependence.

I thank Dr N. C. Fenner for carrying out the mass spectrometric analysis.

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Artificial Semiconductors

In 1968 while spending a sabbatical period at the Allen Clark Research Centre I worked on an idea for making artificial semiconductors and did a few preliminary experiments. It seems worth recording these because, due to the interest of colleagues, we are about to continue the work; and a comparable scheme has been proposed by distinguished scientists at IBM[1].

The basic idea is to make a multilayered structure of alternate thin films of metal and insulator. The resulting potential diagram (Fig. 1) is similar to the Kronig-Penney[2] representation of a crystalline solid. In a solid, of course, the potential barrier is the effort an electron has to make to get from one atom to the next and its width is entirely governed by the crystal lattice spacing, a very inflexible parameter. Because nearly all lattice spacings are between 3 and 5 Å, it follows, from solving the Schrödinger equation with barriers of this periodicity, that the energy spread of a band is a few electron volts. The limits of the band occur when the periodic distance, $(a+b)$ in Fig. 1, is an integral number of half de Broglie wavelengths for an electron.

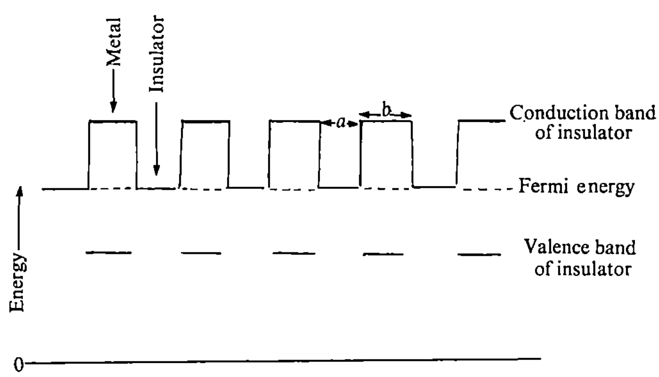

Fig. 1 Potential diagram of a multilayered structure.

If one applies this model to dimensions that are just practicable for an artificial semiconductor, say, sputtered layers of 20 to 30 Å, a great difference occurs. The solution of the Kronig-Penney problem simplifies to an approximate equation

$$\alpha_n a = K \pm n\pi$$

where $\alpha_n = (2mE_n)^{1/2}/\hbar$, E_n is the energy of the nth allowed level, a is the thickness of the metal and K is a constant for a particular structure.

The values of α_n represent allowed energy levels. The allowed band is very narrow, a physical consequence of the wide barrier which holds the electrons to the metal layers. Consequently, once the values of the Fermi level of the metal and the energy gap of the insulator are known it is easy to calculate the energy gap corresponding to, say, the transition from $n=0$ to $n=1$. For a Au–SiO_2 multilayer with $a=30$ Å this comes to about 1.0 eV.

In the experiments I was associated with in 1968 we used an r.f. sputtering set with a rotating electrode, one half SiO_2 and the other half Au. Each side could be made to sputter on to substrates separately. The sputtering rates were determined in the usual way by measuring long exposures, then the times were scaled down so that a thin layer of each material was obtained. Typical runs were made in which about twenty layers each of Au and SiO_2 were sputtered on to glass or 'Irtran' substrates.

Of course a 30 Å layer even by r.f. sputtering must have many voids and islands. Electrical resistance measurements on a forty-four layer structure, showed an ohmic characteristic with a resistance of only $\sim 10^{-2}$ Ω. This was presumably the path of lowest resistance through the layers and may well have been shunting some more interesting characteristics. A more promising diagnostic method is infrared absorption. At the infrared wavelength corresponding to an energy gap electrons should be excited across it, with the corresponding absorption of radiation. Two runs of multilayered structure were therefore sputtered on to an 'Irtran' substrate which has a good transmission characteristic. Infrared from a hot source and a grating monochromator was split into two equal beams one of which was transmitted through the specimen and the other through a blank 'Irtran' substrate. The two beams were detected and the difference signal fed on to a pen recorder. The pen recorder was driven by the same motor that operated the monochromator. The first specimen tested was a forty-four layer structure with nominal 30 Å Au separated by nominal 20 Å SiO_2. An absorption edge well above noise level was found at 9.5 μm with smaller edges at 7.9 μm, 4.3 μm and 3.05 μm. The second specimen, which had only eighteen layers, showed less marked effects. The large discrepancy between the calculated value of 1.0 eV and the measured value of 0.13 eV for the principal absorption edge indicates either that the simple theory is inadequate or perhaps that it is wrong to take a value for the bulk Fermi energy of gold (5.6 eV) in considering thin layers. The value of the calculated energy gap is sensitive to this value, decreasing to 0.04 eV if the Fermi energy is zero.

In spite of this limitation of theory the prospect of achieving man-made semiconductors is an exciting one. These structures are difficult to make, so it is too early to be definite about their application. The idea of tailoring a photoconductor for specific narrow band infrared detection is, however, attractive. It is also likely that negative resistance characteristics could be created if a higher uniformity could be achieved. Further experimental and theoretical work is needed to explore these possibilities.

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BIOLOGICAL SCIENCES

Role of ATP in DNA Replication

The bacterial chromosome replicates in a semiconservative manner[1]. This process may occur at the cell membrane[2,3]. Efforts to study replication by means of purified DNA polymerases have failed to elucidate the mechanism of the *in vivo* process. Therefore, recent attempts have been made toward isolation of membrane-containing systems which more nearly approximate the properties of *in vivo* replication. Two different procedures have been used. One relies on hydrolysis of the cell wall by lysozyme, followed by gentle disruption of the spheroplast membrane so as to avoid shearing the replication apparatus[4,5]. In a different approach, cells have been made permeable to nucleotides and other small molecules by treatment with toluene[6-8] or ether[9]. This permits incorporation studies with little alteration of the cell interior. Both of these procedures yield preparations that replicate bacterial or phage

DNAs semiconservatively in the presence of ATP, as measured by pycnographic analysis of the product[9–11]. In the absence of ATP[6], synthesis appears to be of the repair type[12]. In these systems, ATP does not merely protect the deoxynucleoside triphosphate pool[9,13] or stimulate the rec B, C nuclease[6].

These observations suggest that ATP has some specific role in the replication process. To examine the nature of this role, a membrane preparation has been derived from a wild type strain of *Escherichia coli*, W3110, which exhibits many of the properties of the *in vitro* replicating systems described above. We present evidence that in this system the presence of ATP suppresses the utilization of deoxynucleoside triphosphates for repair synthesis and diverts them to replicative synthesis. In these conditions the incorporation of precursor shows unusual kinetics.

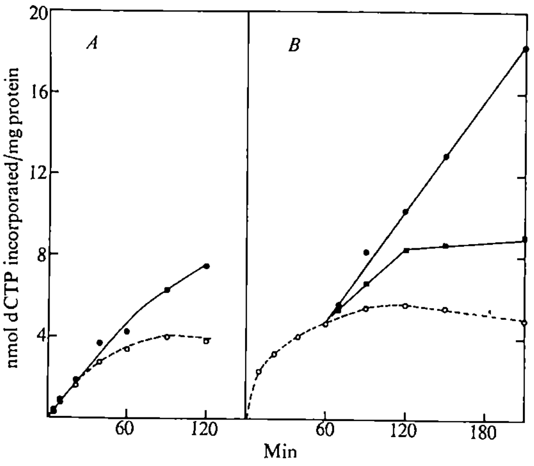

Fig. 1 Kinetics of DNA synthesis by the membrane preparation. *E. coli* W3110 was grown in T-broth[14] to a density of about 5×10^8 cells/ml., washed and resuspended in 0.05 volumes of 0.05 M Tris buffer, *p*H 7.4, containing 20% sucrose. The suspension was treated with 0.005 M EDTA and 50 µg/ml. of egg white lysozyme for 15 min at 30° C. The resulting spheroplasts were sedimented at 10,000*g* in the Sorvall centrifuge for 10 min. The pellet was subjected to two cycles of quick freezing and thawing. The lysed spheroplasts were resuspended in 0.05 volumes of 0.05 M Tris buffer, *p*H 7.4, containing 0.01 M $MgCl_2$ (Mg-buffer) and centrifuged at 1,500*g* for 5 min. The supernatant was again centrifuged at 15,000*g* for 15 min. The pellet was washed once with the same volume of Mg-buffer and resuspended in 0.02 volumes of that buffer (membrane). Unless otherwise stated, 150 to 200 µg of membrane protein was used per ml. of reaction mixture. *A*, The reaction was carried out at 37° C in a mixture containing 55 µM each of dATP, dGTP, TTP and ^{3}H-dCTP (5 µCi/ml.), 8 mM $MgCl_2$, 50 mM Tris-HCl buffer, *p*H 7.8, and 0.33 mM ATP where indicated. Aliquots of 0.1 ml. were withdrawn and dried on 2.3 cm Whatman No. 3 filter paper disks, which were washed several times with 0.3 M trichloroacetic acid, once with acetone and then dried. The dried disks were transferred to vials containing 5 ml. of toluene-based scintillation fluid and counted in a scintillation spectrometer. *B*, ATP (0.33 mM) or deoxynucleoside triphosphates (25 µM each) including ^{3}H-dCTP (2.3 µCi/ml.) were added to different aliquots of a minus-ATP reaction mixture at 60 min. ●, Plus ATP; ○, minus ATP; ■, plus deoxynucleoside triphosphates.

The incorporation of ^{3}H-dCTP into DNA by these membrane preparations continued for 40 to 60 min, and then ceased (Fig. 1*A*). In the presence of ATP the reaction continued for at least another hour. If ATP was added 60 min after the start of a reaction, when incorporation of ^{3}H-dCTP had ceased, incorporation resumed immediately and continued at a linear rate for 2 h (Fig. 1*B*).

Sustained DNA synthesis in the presence of ATP may result from the regeneration of deoxynucleoside triphosphates that are hydrolysed during the reaction. To examine this possibility, aliquots were taken at several times throughout the course of a reaction and analysed by thin-layer chromatography for the fate of ^{3}H-dCTP. The results are shown in Fig. 2*A*. By 60 min only 14 nmol of the original 50 nmol of triphosphate remained in the reaction lacking ATP, whereas 25 nmol of triphosphate was present in the reaction containing ATP. However, when an additional 25 nmol of each of the four triphosphates was added to a reaction mixture lacking ATP at 60 min to replenish the substrates, incorporation resumed, but at only 60% of the rate attained when ATP was added and for only 60 min (Fig. 1*B*). Furthermore, the addition of 50 nmol of the four triphosphates did not increase the rate of incorporation beyond that observed with 25 nmol. Thus, although ATP did protect the deoxynucleoside triphosphate levels to some extent, it may have an additional function that cannot be replaced by the deoxynucleotides.

To examine this possibility further, the effect of the α,β-methylene analogue of ATP was studied. When added to a reaction mixture containing ATP, the analogue did not prevent the ATP-induced protection of ^{3}H-dCTP (Fig. 2*A*). However, it completely prevented the sustained incorporation of ^{3}H-dCTP produced by ATP (Fig. 2*B*). Therefore, the analogue seems to separate the requirement of ATP for triphosphate regeneration from some additional role in DNA synthesis. We shall now show that ATP has a qualitative effect on DNA synthesis, beyond its ability to maintain adequate levels of the deoxynucleoside triphosphates.

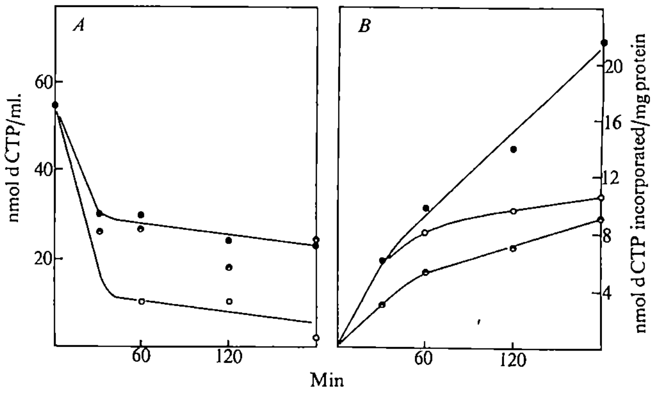

Fig. 2 Effects of ATP and its α,β-methylene analogue on deoxynucleoside triphosphate level and DNA synthesis. *A*, Degradation of ^{3}H-dCTP. The incubation mixture was the same as described in the legend to Fig. 1 except that ATP (0.33 mM) and α,β-methylene ATP (1.0 mM) were added as indicated below. Aliquots of 0.05 ml. were withdrawn and deproteinized by treatment with 0.3 M perchloric acid after adding 100 µg of bovine serum as carrier. The precipitate was removed by centrifugation. The supernatant was neutralized by KOH and analysed by PEI-cellulose thin-layer chromatography for nucleotides. The chromatogram was developed for 2 h with 0.1 M acetic acid–1.2 M LiCl. *B*, Incorporation of ^{3}H-dCTP. The incubation mixture was the same as in *A*. Incorporation of radioactivity into DNA was measured as described in Fig. 1. ●, Plus ATP; ○, minus ATP; ◓, plus ATP and α,β-methylene-ATP.

Sulphydryl compounds inhibit replicative synthesis but not repair synthesis in permeabilized cells and membrane preparations that require ATP for semiconservative DNA replication[5–7]. In our system (Table 1), N-ethylmaleimide (NEM) slightly stimulated ^{3}H-dCTP incorporation in the absence of ATP, but inhibited incorporation by about 65% in the presence of ATP. Since the initial rate of DNA synthesis in the presence and absence of ATP was the same (Fig. 1*A*), these results indicate that ATP induces the NEM-sensitive replicative type of DNA synthesis and simultaneously suppresses the NEM-insensitive repair type of synthesis in these preparations.

To characterize further the nature of the reaction, the product synthesized in a reaction mixture in which dBrUTP replaced

TTP was analysed pycnographically. It was found that more than half of the product showed a hybrid density when ATP was included in the reaction (Fig. 3). Absence of ATP resulted in the synthesis of light DNA only. These results show that ATP is essential for semiconservative replication in this system and that it can partially suppress the repair type of DNA synthesis.

ATP may act at more than one step in the process of DNA replication. We examined the assumption that one role of ATP is to alter some component required for DNA replication, either substrate, primer or enzyme, which is common to both the repair and replicative systems. This process might be observed as a brief interval during which the labelled deoxynucleoside triphosphate cannot be utilized. Fig. 4 shows that when ATP and ^{3}H-dCTP were added to a membrane preparation after 40 min of DNA synthesis in the absence of ATP, label was not incorporated into acid precipitable material for about 100 s. By contrast, in the control experiment where no ATP was added with ^{3}H-dCTP, no lag of incorporation could be detected. A shorter lag of 20 to 30 s was observed when the ATP and ^{3}H-dCTP were added at the very start of synthesis. The longer lag at 40 min may result from the much lower rate of repair synthesis occurring at that time (Fig. 1). In reaction mixtures containing purified DNA polymerase I in place of the membrane preparation, ATP induced no lag of incorporation, showing that the presence of ATP did not directly prevent utilization of deoxynucleoside triphosphates.

In an effort to determine the basis for this lag of incorporation of ^{3}H-dCTP, we tested for the presence of this nucleotide 150 s after its addition to a reaction mixture like that shown in Fig. 4. The mixture was analysed by DEAE-'Sephadex' chromatography. Fig. 5*A* shows that more than 60% of the

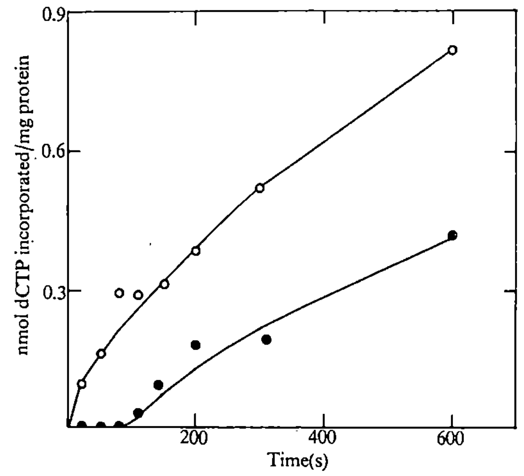

Fig. 4 Effect of ATP on the incorporation of ^{3}H-dCTP. A reaction mixture like that described in the legend to Fig. 1, containing four deoxynucleoside triphosphates but no ATP or ^{3}H-dCTP, was incubated at 37° C for 40 min. ATP (0.33 mM) and ^{3}H-dCTP (20 μCi/ml.) were then added. Aliquots of 0.10 ml. were withdrawn at indicated time intervals and prepared for counting. ●, ATP added with ^{3}H-dCTP; ○, ^{3}H-dCTP added alone (no ATP).

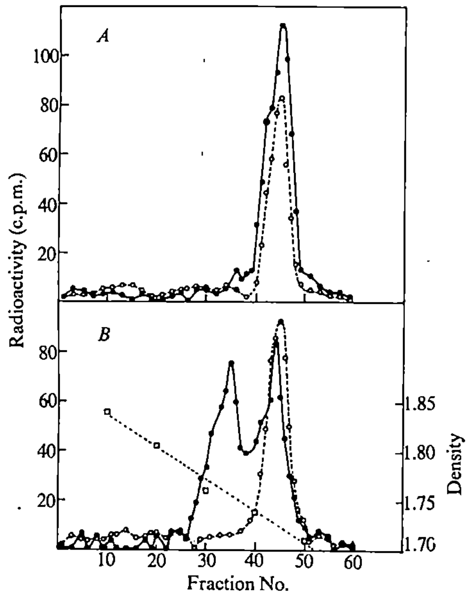

Fig. 3 Pycnographic analysis of the product. The incubation was carried out at 37° C for 20 min in a reaction mixture like that described in the legend to Fig. 1 except that TTP was replaced by dBrUTP, either (*A*) in the absence of ATP, or (*B*) in the presence of ATP. Aliquots of 0.3 ml. of the reaction mixture were mixed with ^{32}P-labelled *E. coli* DNA and 0.10 ml. of 1.5% 'Sarkosyl'-0.05 M Tris-HCl, *p*H 7.3–0.01 M EDTA. The clear mixture was then sheared according to the method of Burger[15], mixed with 4.5 ml. of CsCl (density 1.808 g/cm^3) and centrifuged in the Spinco rotor SW 39 at 36,000 r.p.m. for 64 h at 22° C. Fractions of two drops were collected directly on filter paper disks. The density of selected fractions was determined refractometrically. The paper disks were treated and counted as described in the legend to Fig. 1. ●, Product; ○, marker ^{32}P-labelled *E. coli* DNA; □, density.

Table 1 Effect of NEM on DNA Synthesis by Membrane Preparations

Reaction mixture	nmol ^{3}H-dCTP/mg protein/20 min
Complete (+ATP)	1.944
Complete+NEM (20 mM)	0.667
Complete−ATP	1.888
Complete−ATP+NEM (20 mM)	2.611

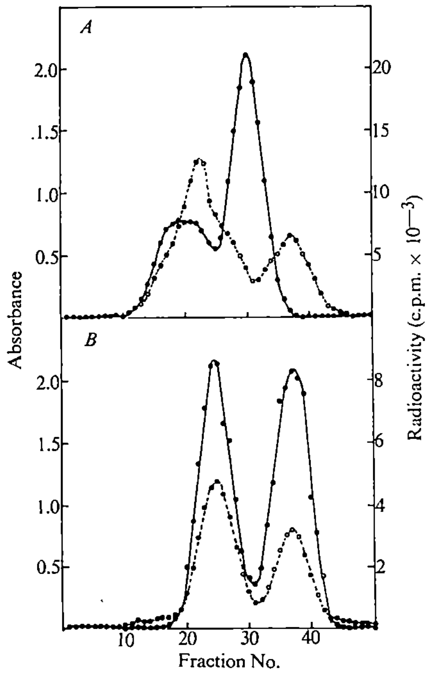

Fig. 5 Analysis of the reaction mixture by column chromatography. The incubation was carried out as described in the legend to Fig. 4, but with 400 μg of membrane protein per ml. of reaction mixture. After addition of ATP (0.33 mM) and ^{3}H-dCTP (100 μCi/ml.) the mixture was incubated for 150 s and then chilled. The material was mixed with dCTP and dCDP and adsorbed to a column of DEAE-'Sephadex A-50'. The adsorbed nucleotides were eluted and rechromatographed with a linear gradient from 0 to 0.25 M KCl in 0.05 M Tris-HCl, *p*H 7.9. Fractions of 1.2 ml. were collected. *A*, Plus ATP. *B*, Minus ATP. ●, Radioactivity; ○, absorbance at 260 nm.

radioactivity eluted from the column at a position intermediate between dCDP and dCTP with little material at the position of the original triphosphate. In contrast, no material was found at this elution position when the reaction was carried out in the absence of ATP (Fig. 5*B*). The large amount of dCDP in the control reaction probably resulted from the high concentration of membrane protein that was used in these experiments. Examination of the altered material by sucrose gradient sedimentation showed that it did not sediment faster than pure ^{3}H-dCTP. This indicates that its altered chromatographic position did not result from binding to macromolecular components of the reaction mixture. It is not yet clear whether the other three deoxynucleoside triphosphates experience a similar alteration.

Thus, the membrane preparation from *E. coli* W3110 exhibits two of the types of DNA synthesis that are observed *in vivo*—repair and replication. ATP seems to play a key role in the control of these reactions. In the absence of ATP, synthesis occurred only by the repair mechanism. This process was insensitive to NEM and resulted in an imperceptible change in density of the template DNA when density-labelled precursor was present in the reaction mixture. The results indicate that ATP suppressed this type of synthesis. In the presence of ATP, incorporation of label occurred at the same rate but was inhibited by NEM by more than 65%, and more than half of the product showed a hybrid density after synthesis in the presence of density-labelled precursor. This indicated that more than half of the synthesis was due to extensive semiconservative replication.

ATP may regulate the relative activity of these alternative mechanisms of DNA synthesis by altering some component involved in the replication process. This would account for the 100 s lag before incorporation of labelled deoxynucleoside triphosphate into DNA when ATP was present, and immediate incorporation when ATP was absent.

It is likely that the synthesis of a short polyribonucleotide primer is essential for the initiation of DNA replication. Isolation of replicating DNA covalently linked to small polyribonucleotide fragments[16], and the requirement of RNA synthesis before the onset of DNA replication in several systems[17–19], support this idea. Synthesis of such primer polyribonucleotide could block the site for repair synthesis resulting in the transient halt of both synthetic activities. In our system ATP is the most efficient of the ribonucleoside triphosphates for stimulating DNA synthesis, and a mixture of all four ribonucleoside triphosphates did not stimulate the formation of hybrid DNA more than did ATP alone. This suggests that the putative polyribonucleotide primer is predominantly polyadenylic acid.

The alternative explanation for the role of ATP is that it modifies the deoxynucleoside triphosphates and that this explains the incorporation lag shown in Fig. 4. It remains to be shown whether this modification of dCTP is an important or trivial aspect of the role of ATP in DNA replication.

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Specificity and Molecular Features of an Insect Attractant in a *Drosophila* Mutant

SEVERAL attractants and repellents which are highly specific for a given species or sex have been identified in the last decade. Although much effort has been concentrated on demonstrating the molecular basis of odour perception in insects, the mechanisms involved are still far from clear[1]. As the complicated network of cellular function in chemotaxis has been successfully analysed in bacteria, using a mutation which indicates alteration of one element involved at a time, a similar approach is expected to be fruitful in studies of the olfactory system[2,3]. *Drosophila* is the insect of choice for such studies because of its large stock of marker genes and crossing-over suppressors which are essential for the isolation of induced olfactory mutants. Recent success in the isolation of behavioural mutants of *Drosophila* has thrown light on this subject[4]. I shall describe here the properties of an olfactory mutant of *Drosophila melanogaster* and the molecular characteristics of the odours to which the mutant responded specifically.

Using a potent mutagen for *Drosophila*, ethyl methane sulphonate (EMS), an olfactory mutant (*HPB-1*), showing considerable attractiveness to 4-(*o*-hydroxyphenyl)-3-buten-2-one (HPBO), was isolated from the acetic acid-attractive line (*AA75*) which was originally established from the *Oregon R* wild strain. The details of the isolation of the mutant and its genetic properties will be described elsewhere. The mutation probably involves a dominant gene in the second chromosome.

Behavioural changes in the mutant flies in response to various odours and water were examined using an olfactometer, and compared with those of the parent flies. Flies aged 0–36 h cultured on a corn-yeast medium were starved for 28 h at 25° C. Two hundred and fifty millilitres of freshly prepared odour solution and the same amount of distilled water were each kept in a gas washing bottle at 20° C. Four hundred millilitres of two testing vapours, approximately equal to the capacity of each olfactometer channel, was passed at a rate of 100 ml. min^{-1} for 4 min through two arms of the olfactometer. Tests were done at 25° C in the dark, at a flow rate of 2 ml. min^{-1} for 1 h. The flies trapped in each trapping bulb were cooled and counted. The responses of the mutant flies to given odours were evaluated by determining the ratio of the number of flies trapped in the odour side of the olfactometer to the total number trapped in the two bulbs (R_m), and comparing it with that of parent flies (*Rp*).

Of sixty-four odours examined, the mutant was specifically attracted by twelve chemicals which were repellent to the parent

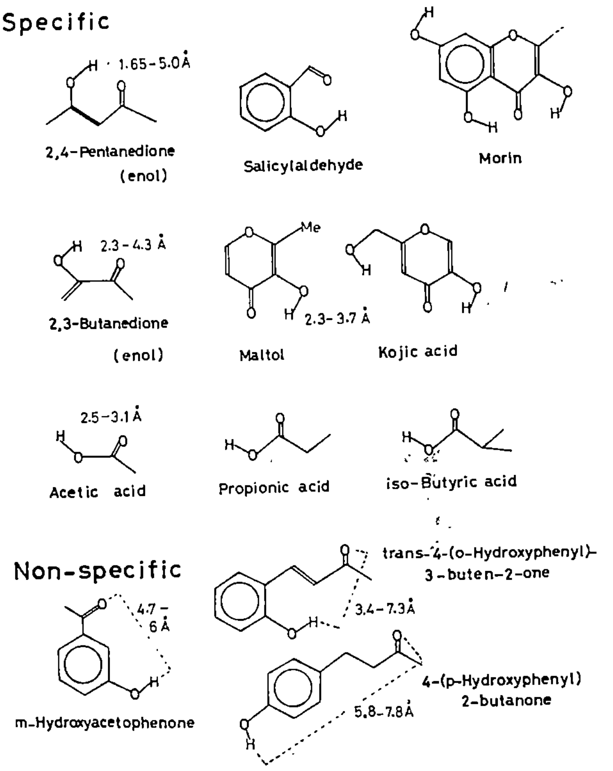

Fig. 1 Comparison of the molecular structures of specific odours with those of non-specific ones. Distances between =O atom and −OH proton were estimated by the chemical model, W. Büchi_Glasapparatefabrik Flawil.

strain (*AA75*). All the other fifty-two odours were non-specific. (Odours are defined as specific where R_m–R_p >15%.) Table 1 shows that these twelve specific odours, included a variety of chemicals: α and β-diketones (2,3-butanedione and 2,4-pentanedione), aliphatic monoketones (2-propanone, 3-heptanone and 5-nonanone), γ-pyrones (kojic acid and maltol), a flavon (morin), an aldehyde (salicylaldehyde), monocarboxylic acids (acetic acid, propionic acid and *iso*-butyric acid). The results obtained so far indicate that certain functional groups such as methyl, methoxyl and phenyl do not play an important role in producing the present specificity; nor do molecular size or shape seem to be relevant. Hydroxyl, carbonyl, aldehyde and carboxylic groups, on the other hand, appear to effect the specificity. The structure of each of the seven specific odours, maltol, kojic acid, morin, salicylaldehyde and three carboxylic acids, includes a proton acceptor (=O) and a proton donor (–OH), the minimal distances between which are 1.65 to 2.5 Å. Furthermore, in the gas phase 2,4-pentanedione apparently assumes the enol form (almost 90%) enolization[5], forming a structure similar to salicylaldehyde and morin. The α-diketone, 2,3-butanedione, contains about 0.6% of the enol form in the absence of solvent[6], forming a structure similar to maltol and kojic acid. So these nine chemicals contain any one of the following three structures (Fig. 1): (1) the 2,4-pentanedione type, including salicylaldehyde and morin where the distance between the =O atom and –OH proton (A–D distance) ranges from 1.65 to 5.0 Å, giving an average value ($\overline{\mathrm{AD}}$) of about 3.3 Å; (2) the 2,3-butanedione type including kojic acid, maltol and morin where the A–D distance is 2.3–3.7 Å for γ-pyrones and flavon ($\overline{\mathrm{AD}}$: 3.0 Å) and 2.3 to 4.3 Å for 2,3-butanedione ($\overline{\mathrm{AD}}$: 3.3 Å); and (3) the acetic acid type, including three carboxylic acids the A–D distance of which ranges from 2.5 to 3.1 Å ($\overline{\mathrm{AD}}$: 2.8 Å).

My assumption that the "bifunctional unit" plays a part in the present specificity is supported by the following facts: (1) None of ten alcohols and phenols such as ethanol, pyrocatechol, *o*-hydroxybenzyl alcohol, 4-methyl-2-pentanol and nerol showed specificity. (2) All six chemicals in which the average A–D distance ($\overline{\mathrm{AD}}$) ranges from 5.3 Å (dimedone, enol[7]) to 6.9 Å (*p*-hydroxypropiophenone) were entirely non-specific. (3) Of six chemicals having two proton acceptors, the average distance between the two oxygen atoms of which ranged from 3.1 Å (2,5-hexanedione and dimethylsuccinate) to 5.2 Å (dimethylfumalate), none was specific for the mutant.

On the other hand, in the three monoketones (2-propanone, 3-heptanone and 5-nonanone), which are in the specific odour group, absorption was shown by infrared spectrometry to be attributable to the hydroxyl group. This implies that the present specificity is not a consequence of impurities having a hydroxyl group, but of the chemicals themselves. One explanation based on the "bifunctional unit model" is, however, possible if it is assumed that there are two molecules of enol and keto forms which fulfil the role of proton acceptor and donor. But knowledge of the keto-enol equilibria of these chemicals in gas phase is vague. The last mentioned chemical, 4-(*o*-hydroxyphenyl)-3-buten-2-one ((Eastman Organic Chemicals, USA), which was the first to be described as a specific odour and showed the highest degree of specificity among chemicals examined (Table 1), contained a *trans* isomer as its chief constituent and an unknown trace; both of them, however, turned out to be non-specific. Although the *cis* isomer certainly has the characteristic structure where the A–D distance is 0.8–2.6 Å (neglecting the interatom repulsion), it is not yet conclusively known whether the present specificity is attributable to the *cis* isomer, as the latter has not been isolated. In addition a nuclear magnetic resonance experiment (NMR) gives no signal which can be attributed to the *cis* configuration. If the "bifunctional unit" is a common feature of the odour molecule of different specific compounds it follows that there may be a particular receptor site, also constituting a bifunctional unit, of a similar nature and that the interaction between the odour molecule and the receptor site is due to two simultaneous hydrogen bonds. Several compounds with specific odours, such as 2,4-pentanedione, morin, salicylaldehyde and acetic acid, are well-known chelating agents for heavy metals. It has been suggested that certain heavy metals could play a part in olfactory perception of the house fly, as injection of the metal chelating agent 'Nitroso-R' causes changes in response to *n*-butanol[8].

Table 1 Responses of Mutant and Parent Flies to Odours

Odour	Concentration (M)	Response (%)* Parent (R_p)	Mutant (R_m)	Difference (%) (R_m-R_p)
2,4-Pentanedione	10^{-1}	35	58	23
Salicylaldehyde	10^{-3}	40	63	23
Morin	2×10^{-4}	43	61	18
Maltol	10^{-3}	39	60	21
Kojic acid	10^{-3}	35	55	20
2,3-Butanedione	10^{-2}	39	62	23
Acetic acid	10^{-3}	27	53	26
Propionic acid	10^{-1}	39	69	30
iso-Butyric acid	10^{-2}	42	59	17
2-Propanone	4×10^{-1}	52	79	27
3-Heptanone	10^{-5}	34	57	23
5-Nonanone	10^{-4}	21	53	32
4-(*o*-Hydroxyphenyl)-3-buten-2-one	2×10^{-4}	33	71	38
Diacetone alcohol	10^{-1}	37	37	0
Phthiocol	10^{-4}	86	82	−4
3-Hydroxyflavon	10^{-5}	78	73	−5
Tropolone	10^{-1}	44	47	3
m-Hydroxyacetophenone	2×10^{-3}	73	68	−5
4-(*p*-Hydroxyphenyl)-2-butanone	5×10^{-4}	76	70	−6
trans-4-(*o*-Hydroxyphenyl)-3-buten-2-one	2×10^{-4}	58	54	−4

* Represented as the ratio of the number of flies trapped in the odour side of olfactometer to the total number of flies trapped in the odour and water side. No. of tests, 113; No. of flies trapped, about 28,500.

A variety of chemicals which are definitely non-specific have the bifunctional unit. These include the 2,4-pentanedione type: *o*-hydroxyacetophenone and *o*-hydroxypropiophenone; the 2,3-butanedione type: 3-hydroxyflavon and phthiocol; the acetic acid type: fumaric acid and several carboxylic acids. Other non-specific odours, such as tropolone, purpurogallin, diacetone alcohol and acetoin, may belong to a similar category, as they have a feature closely resembling the bifunctional unit. It seems then that there are still unknown features which have certain functions in the interaction between the odour molecule and the receptor site.

A characteristic feature of the present mutation is that the mutant was strongly attracted by chemicals which repelled the parent strain. The behaviour of the mutant might be explained if it contains a missense mutation producing a modification or defect in a receptor site which interacts with the repellents or in the mutant's nervous system. The fact that the mutant is strongly attracted by chemicals which repel the parent flies implies that there is at least a different site, and that the neural excitation thus produced leads after integration to an orientated movement towards the odour source. This indicates that odours act on different sites rather than on any particular one. Thus, the results obtained so far suggest that the use of olfactory mutants could lead to the identification of receptor sites and to the isolation of substances involved in the olfactory system. Finally, the use of such mutants should be of help in analysing the complicated network of the olfactory system.

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Collection, Motility and Storage of Spermatozoa from the African Elephant *Loxodonta africana*

NATURAL breeding of elephants in zoos is uncommon because bulls have proved dangerous to handle and are not usually kept. The collection and storage of elephant semen and artificial insemination are, therefore, being investigated by the Zoological Society of London as part of a research project on the preservation of mammalian spermatozoa[1]. Problems of semen collection[2] were simplified by taking advantage of the culling programme in progress at the Kruger National Park, South Africa.

Mature bull elephants, 15 to 50 yr old, were immobilized by shooting with darts that injected 'Scoline' (Glaxo Laboratories Ltd) on impact, then bled from the jugular vein and eviscerated. The reproductive tract was removed within 15 min of death and the excurrent duct of the testis dissected. Spermatozoa were removed by flushing the duct posteriorly. There were many more in the distal region and they showed such structural signs of maturity as a condensed acrosome and a distally-situated protoplasmic droplet. These characteristics were absent in spermatozoa recovered from the proximal portion of the duct.

The report of Short *et al.*[3] that spermatozoa removed from the distal portion of the duct were immotile was confirmed; only one sample examined before dilution showed any signs of activity and in this only 15% of spermatozoa showed weak flagellation. Various methods of inducing motility in these spermatozoa were tested. Subsamples (0.03 ml.) of effluent flushed from the duct with air were diluted into 15 ml. tubes containing 2.0 ml. of buffered saline (5 mM sodium phosphate and 149 mM sodium chloride, *p*H 6.8) alone, or including 5 mM potassium chloride or 10 mM fructose or both substances. Some samples were examined with a field microscope to confirm that the spermatozoa were motile immediately after dilution. Tubes were stoppered and transferred in a vacuum flask, at 30 to 35° C, to a temporary laboratory. After storage for 2 to 3 h they were examined microscopically at ambient temperature (20 to 25° C) and motility was observed in all samples. Table 1 shows mean scores of the rate of progressive motility[4] (range 0 to 4) and percentage of motile spermatozoa. The addition to the diluent of potassium, fructose, or both increased the proportion of motile spermatozoa ($P<0.05$).

Table 1 Mean* Scores of Viability of Elephant Spermatozoa in Various Diluents and after Incubation for 3 h at 30° C

Diluent	Motility score	Motile spermatozoa (%)
Buffered saline	1.8	30
Buffered saline + KCl	2.3	40
Buffered saline + fructose	2.3	40
Buffered saline + KCl + fructose	3.3	50
Standard error of means †	0.60	4.7

* Results are means of samples from three elephants.
† From residual mean square in analyses of variance.

Several experiments were also carried out to determine a suitable method of freezing spermatozoa. Spermatozoa were flushed from the duct with a diluent which consisted of a 1 : 1 mixture of egg yolk and buffered citrate. The latter contained 20 mM sodium phosphate and 80 mM sodium citrate (*p*H 6.8). The flushings were then further diluted with the egg yolk citrate to achieve a rate of approximately four-fold (approximately 1×10^9 spermatozoa ml.$^{-1}$) and cooled to 5° C for 2 to 3 h. They were then diluted 1 : 1 with the buffered citrate containing a protective agent (dimethyl sulphoxide (DMSO) or glycerol), equilibrated for at least 0.5 h and then frozen in 0.5 ml. Cassou[5] straws over liquid nitrogen (−196° C). Frozen samples were stored for at least 24 h before thawing. The efficacy of the two protective agents at final concentrations of 2, 4, 8 and 16% v/v was compared in a 2×4 factorially designed experiment, replicated using spermatozoa from four elephants. As judged by scores of progressive motility, the percentages of motile spermatozoa and unstained spermatozoa in eosin-nigrosin smears[6], DMSO was more effective than glycerol and the best concentration of DMSO seemed to be 8%. Egg yolk-citrate diluent containing DMSO was better than egg yolk-glucose diluents[7] containing glycerol or DMSO; a combination of 7% DMSO and 1% glycerol in egg yolk-citrate was even better than the diluent containing 8% DMSO. The combination was used to prepare a bank of deep-frozen spermatozoa; Table 2 gives details of the quality of the elephant semen samples stored in this bank after transport to, and storage for, 3 weeks at the Wellcome Institute in London.

Attempts were also made to collect semen from live elephants following electrical stimulation. On two separate occasions, a bull elephant was immobilized with 'Immobilon' (Reckitt and Colman, Hull) and stimulated with a rectal probe using a variable output square wave stimulator powered by a 12 V battery. The probe was 66 cm long and 13 cm in diameter and consisted of four sets of electrical contacts, each set comprising four brass plates having surface dimensions of 2 × 2.5 cm. The four plates in each set were equally spaced around the circumference of the probe, which was wired so that a ring stimulus of bandwidth of 2.5 cm could be obtained in any one of four positions by bridging adjacent quadrants. The first position was 10 cm below the apex of the probe and positions 2, 3 and 4 were situated posteriorly and separated by spaces of 2 cm. The positions for optimal stimulation were found to be 1 and 2, when the probe was fully inserted into the rectum, using an output of 6 to 10 V, a pulse-frequency of 30 Hz and a duration of 12 ms. An erection occurred in the first elephant and, within 20 min, 80 ml. of a translucent viscous liquid had been collected which did not contain spermatozoa; the battery was suspected. A new battery was substituted and 260 ml. of a fluid of similar appearance was emitted from the second bull in 15 min; more could probably have been collected had the stimulus been continued. The fluid contained 4.8×10^6 spermatozoa $ml.^{-1}$; the motility score was 3.5 (range 0 to 4, ref. 4) and 60% of the spermatozoa were motile.

Table 2 Characteristics of Deep-frozen Elephant Spermatozoa Immediately after Thawing and Viability after Incubation for 1 and 2 h at 30° C

	Scores of viability examined						
	Immediately after thawing			After incubation for			
				1 h		2 h	
Elephant No.	Motility	% motile	% unstained*	Motility	% motile	Motility	% motile
931	3.0	50	85	2.5	50	2.5	40
942	3.0	50	88	3.0	50	2.0	35
945	3.5	60	90	2.5	30	3.0	50
915	1.0	10	68	1.0	10	0.5	10
944	3.0	30	95	3.0	30	1.5	15
907	3.5	60	94	3.0	30	2.5	40
943	3.5	50	93	1.0	30	2.0	30
Mean	2.93	44.3	87.6	2.3	32.9	2.0	31.4
Standard error	0.34	6.3	3.5	0.34	5.2	0.31	5.4

* Percentage unstained (alive) in an eosin-nigrosin smear.

I suggest that elephant spermatozoa are immotile before ejaculation and that dilution can induce motility. As judged by present methods of assessing semen quality, it seems that, with one exception (see Table 2), elephant spermatozoa survived freezing and thawing as well as the deep-frozen cattle semen used at present for artificial insemination. The results of electro-ejaculation are encouraging and will provide a basis for further studies, particularly of the Asiatic elephant the continued existence of which in the wild is in question.

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Paradoxical Effect of Anti-thymocyte Serum on the Thymus

THE suppressive effects of anti-thymocyte serum (ATS) on cell-mediated immune responses[1–4] have been attributed to the selective depletion of recirculating T lymphocytes[5]. In this view, lymphoid tissues such as thymus which do not contain significant numbers of itinerant, blood-borne lymphocytes would be spared from the debilitating effects of ATS. The observation that the rate of recovery from the effects of ATS is substantially accelerated in the presence of an intact thymus[6,7] is consistent with this idea. That the thymus is spared is also supported by studies of the distribution of labelled ATS immunoglobulin (IgG), which indicate that antibody penetrates the thymus very poorly[8]. These considerations do not, however, directly bear on questions concerning possible alteration in immunological activity of thymus cells after the peripheral administration of ATS. The following experiments demonstrate that graft-vs-host (GVH) activity produced by thymus cells is substantially increased for several weeks after the peripheral administration of a single dose of ATS. As this effect was not produced by normal rabbit serum and was removed by absorption of ATS with thymocytes, it seems to represent a specific immunological consequence of administration of ATS.

Table 1 Effect of Administration of ATS on GVH Reactivity of Splenic and Thymus Cells

Days after ATS	Cell No./ thymus ($\times 10^{-6}$)	Spleen index thymus (5×10^6)	Spleen index spleen (5×10^6)
0	105± 6	1.13±0.06	2.49±0.06
1	96±14	0.99±0.03	1.16±0.12
2	98±10	2.37±0.18	1.06±0.08
3	101± 9	2.45±0.14	ND
7	ND	2.08±0.20	1.16±0.11
14	100± 6	2.00±0.16	1.48±0.11
21	101± 8	1.50±0.11	
28		1.34±0.14	1.98±0.19

Rabbit-anti-mouse thymocyte serum was prepared in rabbits according to the method of Levey and Medawar[3]. A pool of serum from eight rabbits was made after determining that each of the individual sera produced at least 90% suppression of the GVH reactivity of spleen cells 2 days after administration. The pooled ATS was inactivated by heating at 56° C for 2 h and absorbed four times with mouse erythrocytes (MRC) at a ratio of one part packed MRC to twenty parts ATS. At various intervals after the subcutaneous injection of ATS into the intrascapular region of Balb/cAnN (H-2^d) mice, GVH activity of various lymphoid tissues was determined in C57Bl/6N × Balb/cAnN (H-$2^{b/d}$)F1 neonatal recipients using a modification[9] of the spleen weight assay described by Simenson[10]. Thymus and spleen were rinsed in Hanks's balanced salt solution and cells gently teased into suspension. Nine days after grafting of cells, recipient litters were killed and the ratio of spleen weight to body weight was determined for both inoculated and control, uninoculated littermates. An index of spleen enlargement (spleen index) was obtained by dividing the spleen/body weight ratio of the injected animals by that

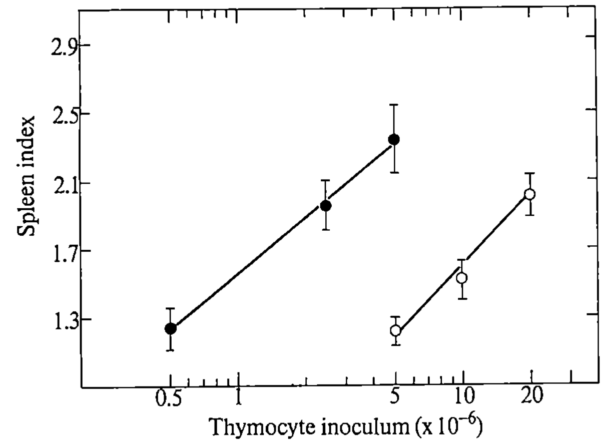

Fig. 1 Graft-vs-host activity of thymocytes obtained from donors given 0.25 ml. ATS subcutaneously 2 days earlier (●), compared with GVH activity of thymocytes obtained from mice given 0.25 ml. NRS subcutaneously 2 days previously (○). Vertical bars denote the limits of one standard error.

Table 3 Enhanced GVH Reactions on Mixing Thymocytes from Animals treated with ATS with Normal Thymocytes

ATS thymus* $(\times 10^6)$	NL thymus $(\times 10^6)$	NL PBL $(\times 10^6)$	Spleen index	Expected spleen index†
0.5			1.26±0.08	
	5.0		1.29±0.14	
0.5	+5.0		2.28±0.26	1.58
		0.20	1.14±0.06	
	5.0	+0.20	2.06±0.18	1.58
0.5		+0.20	1.31±0.19	1.58
5.0			2.37±0.18	
5.0		+0.20	1.84±0.14	2.37

* Thymus from mice given 0.25 ml. ATS 2 days before killing[6].

† By adding individual reactivity from the parallel line assay[12,13].

of their untreated littermates as previously described[9]. In several experiments the relative potency of two different cell suspensions was compared within the same recipient litter by dividing the litter into three groups: recipients of cell suspension 1, recipients of cell suspension 2 and control, uninoculated mice.

A reduction of at least 90% of splenic GVH activity was seen 48 hours after a single injection of 0.25 ml. ATS (Table 1). At the same time, however, a substantial increase in the GVH reactivity of thymus cells was noted. This increased GVH reactivity was present for the following 2 weeks and returned to pre-injection levels by the fourth week, when spleen GVH activity had returned to nearly normal levels (Table 1). It is possible to quantitate the change in GVH reactivity in both tissues after administration of ATS, assuming the activity curves for normal thymus and ATS-thymus are nearly parallel.

Table 2 Effect of Absorption of ATS with Thymocytes on Peripheral Suppressive Activity of ATS and its Enhancing Effect on Thymus GVH Activity

	Spleen index		% Normal reactivity*	
	Thymus	Spleen	Thymus	Spleen
0.5 ml. ATS	1.88	1.06	500	<10
0.5 ml. ATA ā	1.39	1.73	100	25
0.5 ml. NRS	1.26	2.45	N1	N1

* From previously published work[12,13] and this study, using the parallel line assay.

Fig. 1 shows that this parallel relationship obtains at the time of peak reactivity of ATS-thymus; such parallel reactivity lines are seen with almost all tissues[11]. The relative change in GVH activity in both tissues in the 4 weeks after a single injection of 0.25 ml. ATS is shown in Fig. 2. It is apparent that (1) the decrease in splenic GVH activity precedes by approximately 1 day the rise in thymic GVH activity and (2) this dose of ATS seems to have a strikingly reciprocal effect on the GVH activity of these two tissues.

It is unlikely that this effect represents simply the result of a steroid-mediated "stress" reaction and depletion of immunologically irrelevant thymocytes, as the cellularity of the thymus from mice treated with ATS did not differ significantly at any time from the control, uninjected mice (Table 1). To test whether this effect could be accounted for by a non-specific, adjuvant-like property of the rabbit serum, 2 ml. of the ATS was absorbed twice with 5×10^8 Balb/c thymocytes at room temperature for 10 min and compared with (1) ATS which had been absorbed in parallel with 0.1 ml. packed mouse RBC and (2) normal rabbit serum (NRS). Absorption with thymocytes depleted ATS of both peripheral suppressive activity and its enhancing effect on thymus GVH activity (Table 2). Normal rabbit sera produced neither suppressive nor enhancing effects on either tissue.

Previous studies of cells which participate in the donor portion of GVH responses have demonstrated that two types of T lymphocytes can interact synergistically[12–14]. One cell type was found chiefly in the recirculating pool and appeared to amplify the response; the second was enriched in the thymus and spleen and appeared to provide precursors of GVH effector cells. It was therefore of interest to test whether the increased thymic GVH activity could be attributed to an increase in the normally limiting amount of amplifier activity.

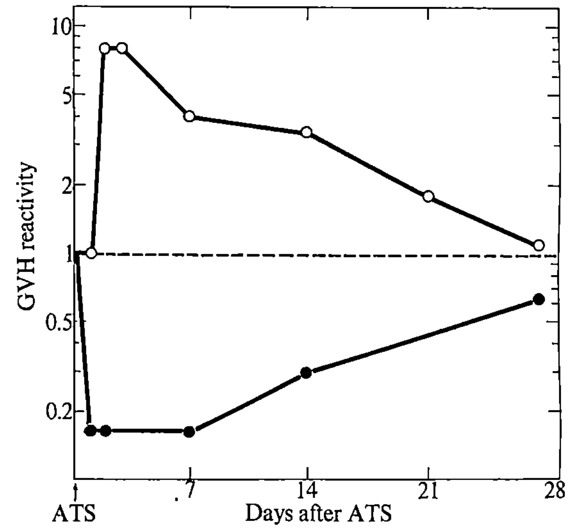

Fig. 2 Comparison of the relative change in GVH activity in thymus (○) and spleen (●) with time after a single subcutaneous injection of ATS (0.25 ml.). ---, Normal.

Mixtures of small numbers of thymocytes from animals 3 days after treatment with ATS with normal thymocytes produced GVH reactions that were substantially in excess of those predicted from the sum of their separate activities (Table 3). Moreover, unlike normal thymocytes, the activity of thymocytes treated with ATS was not significantly enhanced by the provision of small numbers of peripheral blood cells.

In fact, this combination produced a striking antagonistic effect.

These experiments indicate that administration of ATS to mice enhances the GVH activity of the thymus and that this effect is specifically related to the ability of ATS to recognize mouse lymphocytes. The finding is best explained by the idea that administration of ATS provokes an increased rate of differentiation of "immature", immunologically inert thymocytes to "mature" cells which have functional properties in GVH responses that are normally associated with peripheral recirculating T cells, such as the ability to interact synergistically with normal thymocytes in the production of GVH reactions (Table 3).

These experiments do not establish the mechanism responsible for enhanced thymus activity after an injection of ATS. One possibility is that the effect may represent a homeostatic mechanism in which increased intrathymic cell maturation is triggered by depletion of peripheral recirculating lymphocytes, perhaps by the products of ATS-induced lymphocyte destruction. This view receives some support from the observation that GVH hypoactivity in spleen preceded hyperactivity in thymus by as much as 24 h (Fig. 2) and is consistent with the subsequent reciprocal relationship of GVH activity in the two tissues over the following weeks. Alternatively, this effect may be the result of a direct effect of ATS on thymocytes *in situ*, resulting in the stimulation of thymocytes to divide and accelerated differentiation into mature cells. Although this possibility seems unlikely in view of the very poor penetration of labelled ATS into the thymus[8], the demonstration that very low concentrations of some batches of ATS can stimulate lymphocytes to proliferate *in vitro*[15] lends some support to this interpretation.

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1,25-Dihydroxycholecalciferol: Inhibition of Action in Organ-cultured Intestine by Actinomycin D and α-Amanitin

THE stimulatory action of vitamin D on intestinal calcium absorption has been much studied following the pioneering work of Nicolaysen[1] and several important features of the mechanism are fairly well established[2]. Doubt concerning the chemical identity of the biologically active form of vitamin D has arisen, however, leading to extensive investigations of this aspect[3-5]. Two important proposals arose out of this work; first, vitamin D_3 (cholecalciferol) must be first converted to 1,25-dihydroxycholecalciferol (1,25-diOH-CC) (ref. 6) before it can act on the intestine; and second, 1,25-diOH-CC stimulates intestinal calcium transport directly by an actinomycin D-insensitive process[6].

Organ culture of embryonic chick intestine was used to evaluate these proposals. Contrary to the first, vitamin D_3 itself in the culture medium was effective in inducing *de novo* synthesis of the intestinal calcium-binding protein (CaBP) and in stimulating radiocalcium uptake[7,8] and mucosal to serosal transport[9,10]. Here I report results that 1,25-diOH-CC stimulation of the intestinal calcium absorptive mechanism acts through new protein synthesis; both actinomycin D and α-amanitin prevented the response of the organ-cultured intestine to 1,25-diOH-CC.

Table 1 Inhibition of 1,25-diOH-CC Induction of CaBP and ^{45}Ca Uptake in Organ-cultured Chick Duodenum by Actinomycin D

Concentration in medium		CaBP per 100 mg duodenum (μg)	^{45}Ca uptake (% of zero 1,25-diOH-CC control)
1,25-diOH-CC (nM)	Actinomycin D (μg ml.$^{-1}$)		
0	0	0	100
0.65	0	2.73 ± 0.13	133.9 ± 3.9*
0	2 (1.6 μM)	0	100
0.65	2	0	95.3 ± 3.7

Values are the mean ± s.e. Duodena were cultured for 48 h in the presence of 5 mM Ca^{2+}, 1,25-diOH-CC and actinomycin D in the culture medium. The tissues were analysed for CaBP content or transferred to a ^{45}Ca-containing buffer solution and incubated for 30 min at 37.5° C[7]. ^{45}Ca uptake was determined by liquid scintillation techniques on NCS (Amersham/Searle, Illinois) digests of the duodena. Student's *t* test was applied to the uptake data expressed as % dose ^{45}Ca accumulated/100 mg tissue. The uptake data were converted to "percentage of zero 1,25-diOH-CC control" for presentation.

* Treatments resulted in a significant increase in calcium uptake at the 1% level.

The entire duodena from four 20 day old chick embryos were opened and placed mucosa-side-up on a specially designed stainless steel grid[8] inside a Petri dish containing serum-free McCoy's 5A (modified) medium, 2.5–5 mM calcium and 100 units nystatin ml.$^{-1}$. After 48 h in a humidified incubator continuously gassed with an air : O_2 : CO_2 mixture containing 5% CO_2 and 50% O_2, the duodena were either homogenized before CaBP measurement by radial immunoassay[7] or subjected to measurement of radiocalcium uptake during a 30 min incubation in a buffer solution as described previously[7].

Actinomycin D binds to deoxyguanosine residues in DNA, inhibiting DNA-directed RNA synthesis[11]. Vitamin D and its metabolites localize in intestinal mucosal cell nuclei[12,13], and vitamin D stimulates DNA template activity[14] and RNA synthesis[15,16] in the intestinal mucosa, and the vitamin D enhancement of intestinal calcium transport[17,18] is prevented by actinomycin D[17,18]. Vitamin D induces *de novo* synthesis of CaBP[7,19], a protein probably involved in the intestinal calcium absorptive mechanism[9,20], which is inhibited *in vivo* by actinomycin D[21].

When duodena were cultured in the presence of 1,25-diOH-CC (0.65 nM) and 5 mM calcium, CaBP was induced[22], and there was enhanced uptake of ^{45}Ca (Table 1). (Stimulation of ^{45}Ca uptake in organ-cultured duodena can only be seen in response to a medium concentration of 0.65 nM 1,25-diOH-CC after 48 h incubation if calcium is between 2.5 and 5 mM[8].) When 2 µg $ml.^{-1}$ (1.6 µM) actinomycin D was added to the culture medium, however, CaBP synthesis was completely inhibited and ^{45}Ca uptake was not enhanced (Table 1). This was not the result of non-specific toxicity as total protein concentration of the mucosa was not reduced and mucosal structure was intact on histological examination[23] (Fig. 1). I suggest that, like vitamin D_3, 1,25-diOH-CC acts on intestinal calcium transport indirectly by stimulation of DNA dependent-RNA synthesis, and resultant CaBP and/or other protein synthesis, in the intestinal cell.

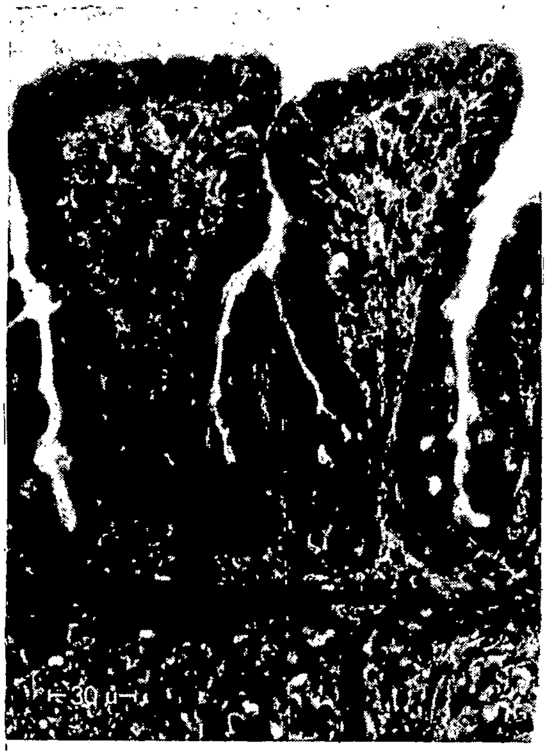

Fig. 1 Mucosal structure of embryonic chick intestine maintained in organ culture for 48 h in the presence of actinomycin D (2 µg $ml.^{-1}$) in the culture medium. Tissues were fixed in a glutaraldehyde buffer, dehydrated in methanol, embedded in glycol methacrylate, 1 µm sections prepared and stained with acid fuchsin-toluidine blue[23].

If intestinal RNA synthesis is essential to the action of 1,25-diOH-CC, then inhibition of DNA-directed RNA polymerase activity would also be expected to inhibit 1,25-diOH-CC induction of CaBP and stimulation of ^{45}Ca uptake as did actinomycin D. α-Amanitin, a cyclic octapeptide isolated from the green mushroom *Amanita phalloides*[24], specifically inhibits nucleoplasmic RNA-polymerase activity in isolated nuclei from sea urchin embryo, rat liver and calf thymus[25,26]. When α-amanitin was included in the culture medium, the activity of 1,25-diOH-CC was inhibited (Table 2); first, CaBP synthesis was completely inhibited at a culture medium concentration of α-amanitin of 0.5 µM; and second, there was complete inhibition of 1,25-diOH-CC enhanced uptake of radiocalcium. This was not due to non-specific toxicity as inclusion of α-amanitin in the ^{45}Ca-containing buffer solution had no effect on 1,25-diOH-CC stimulation of radiocalcium uptake, supporting the conclusion that, like vitamin D_3, 1,25-diOH-CC acts on the intestinal calcium absorptive mechanism by stimulation of RNA and subsequent protein synthesis.

Table 2 Inhibition of 1,25-diOH-CC Induction of CaBP and ^{45}Ca Uptake in Organ-cultured Chick Duodenum by α-Amanitin

Concentration in medium		CaBP per 100 mg duodenum (µg)	^{45}Ca Uptake (% of zero 1,25-diOH-CC control)
1,25-diOH-CC (nM)	α-Amanitin (µg $ml.^{-1}$)		
0	0	0	100
0.65	0	3.8±0.3	137±4*
0	0.5 (0.5 µM)	0	100
0.65	0.5	0	106±3
0	0.5 (in ^{45}Ca buffer only)	—	100
0.65	0.5 (in ^{45}Ca buffer only)	—	136±10*

Medium (Ca^{2+}) was 2.5 mM. See Table 1 for details.

To my belief, my results demonstrate α-amanitin inhibition of sterol induction of a specific protein in an *in vitro* system for the first time. Coupled with the findings of others that α-amanitin inhibited etiocholanolone induction of δ-aminolevulinate synthetase in chick embryo liver *in ovo*[27], and cortisol induction of tyrosine transaminase in rat liver *in vivo*[28], it seems likely that α-amanitin will become important in the study of specific protein synthesis mechanisms in other systems.

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X-ray Photoelectron Studies of Magnesium Ions bound to the Cell Walls of Gram-positive Bacteria

TEICHOIC acids are strongly acidic polymers containing phosphate, which are present in all Gram-positive bacteria[1]. They are found at two distinct locations in the cell envelope: associated with a glycolipid on the outer surface of the cytoplasmic membrane[2,3] and covalently linked to the peptidoglycan of the cell wall[3]. The wall teichoic acid is responsible for the ability of the cell walls of Gram-positive bacteria to bind divalent cations[4] and the membrane teichoic acid mediates an interaction between magnesium ions, bound to the wall, and the cytoplasmic membrane, thus maintaining the optimum cation environment for the activity of magnesium-dependent biosynthetic enzymes in the membrane[5,6]. It is therefore important to identify the interactions involved in binding magnesium to teichoic acids in the bacterial envelope; although electrostatic interaction between the cations and negatively charged phosphate groups is a prominent feature, interactions with other components of the envelope cannot be ruled out. For example, the amount of D-alanine esterified to the wall teichoic acid of *Staphylococcus aureus* H and *Micrococcus* sp. 24 influences the capacity of these walls to bind Mg^{2+} (ref. 4).

Table 1 Composition of Preparations

	Phosphorus as % of total dry wt	Esterified alanine/ phosphate group	μeq Mg/ μmol phosphate
Bacillus licheniformis walls	1.33	0.50	0.36
Bacillus licheniformis wall teichoic acid from hydroxylamine-treated walls	12.1	0.02	0.95
Lactobacillus plantarum walls	3.0	0.75	0.30
Lactobacillus plantarum walls after treatment with hydroxylamine	2.8	0.25	0.44

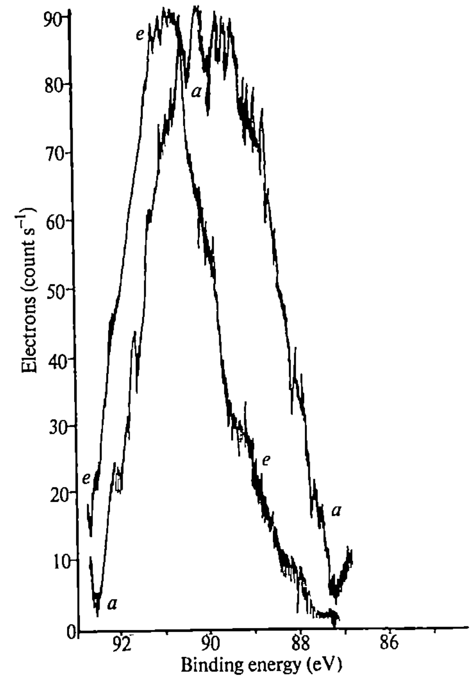

Fig. 1 X-ray photoelectron peaks for magnesium 2s electrons in combined sample of $MgCl_2$ and $MgHPO_4.3H_2O$. *a*, With both salts exposed; *b*, with principally the chloride exposed.

Here we report a study by X-ray photoelectron spectroscopy (ESCA) of samples of cell wall. The samples were examined as powders mounted on double sided 'Sellotape' which was attached to the copper sample block, and were calibrated by reference to the gold $4f_{7/2}$ level electrons (binding energy 84.0 eV) from gold powder on the surface of the specimens. The calibration was done in a separate experiment because the gold electrons interfered with the magnesium 2s electrons; the cell wall nitrogen 1s peak was used as a reference. The spectra were studied using an AEI ES100 X-ray photoelectron spectrometer with Al $K\alpha_1\alpha_2$ radiation. Overlapping peaks (electron count against binding energy) were deconvoluted using a Dupont curve analyser.

We have examined the walls of *Bacillus licheniformis* ATCC 9945 and *Lactobacillus plantarum* ATCC 10241 Mutant R1 (ref. 7). *B. licheniformis* was grown in a medium previously described[5] and *L. plantarum* in the medium described by Douglas and Wolin[7]. Cells were disrupted with No. 11 Ballotini beads in a Braun cell disintegrator and the walls were recovered by centrifugation and washed with 0.9% NaCl and then water. The walls were heated at 100° C for 10 min in order to destroy autolytic enzymes and then washed repeatedly in water and freeze dried. Samples (50 mg) of walls were suspended in 10 ml. of 0.1 M $MgCl_2$, *p*H 6.0, for 1 h at room temperature, recovered by centrifugation at 20,000*g* for 15 min, washed four times with water to remove unbound cations and then freeze dried. Bound Mg^{2+} could be removed from these preparations by suspension in 10 ml. of 0.05 M sodium EDTA, *p*H 6.5, for 30 min at room temperature, followed by centrifugation and washing. Alanine ester residues were removed from the teichoic acid in the walls by treatment with 0.4 M hydroxylamine (salt free)[4]. Wall teichoic acid was obtained by extraction with 10% trichloroacetic acid[6] from walls of *B. licheniformis* which had been treated with hydroxylamine. It was converted to the magnesium salt by repeated passage through a column (1 cm × 10 cm) of 'Dowex' 50 resin in the Mg^{2+} form and freeze dried. Table 1 shows the compositions of these preparations and Table 2 the measured binding energies of the compounds. Alanine was determined with ninhydrin[8] after removal from the wall in 0.1 M NaOH, 37° C, 1 h.

Table 2 Measured Binding Energies

	Binding energy (eV)* Mg 2s	N 1s	P 2p
Bacillus licheniformis walls	90.8, 89.7	398.7	134.7
Bacillus licheniformis wall-teichoic acid from hydroxylamine-treated walls	88.8	399.9	133.3
Lactobacillus plantarum walls	90.6, 89.6	399.4	—
Lactobacillus plantarum walls after treatment with hydroxylamine	89.3	399.7	134.35
Magnesium chloride	90.7		
Magnesium phosphate ($MgHPO_4.3H_2O$)	89.6		134.1

* Probably correct to ±0.3 eV.

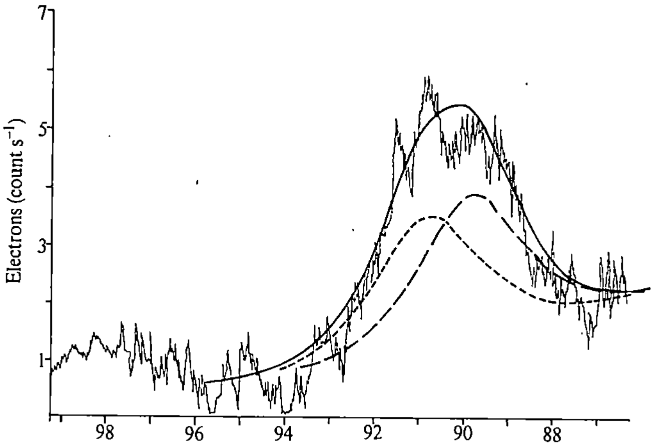

Fig. 2 X-ray photoelectron peak for magnesium 2s electrons in the cell wall of *Bacillus licheniformis*, showing the possible resolution of the broad peak into two component curves.

In the charge potential model[9] the binding energy of an electron can be expressed in terms of the charge on the atom (Q) and the Coulomb potential energy (V) of an electron due to the other atoms of the molecule. Thus

$$\text{Binding energy} = \text{constant} \times Q + V + \text{constant}$$

In an ionic compound the term V includes the contributions from other ions and is therefore a Madelung term. Thus the difference (1.1 eV) in the binding energies of the 2s electrons of Mg^{2+} in magnesium phosphate and magnesium chloride is due to the difference in the Madelung terms for the two ionic compounds. Because this term represents interionic interactions in the ionic lattice we can treat a lower electron binding energy as an indication of a strong interaction of the Mg^{2+} with the anions in the crystal lattice. Because the accuracy of measurement of the binding energies is only claimed to be ±0.3 eV, the small difference between the Mg^{2+} 2s electron binding energies in the two salts was demonstrated with a single rectangular sample one half of which was covered with $MgCl_2$ and the other half with $MgHPO_4.3H_2O$. The resulting broad peak is shown in Fig. 1*a*. When the sample was partly retracted to expose more of the chloride section and less of the phosphate a narrow peak at higher binding energy, due to the chloride Mg 2s electrons, with a small shoulder at lower binding energy, due to the phosphate Mg 2s electrons, were observed (Fig. 1*e*).

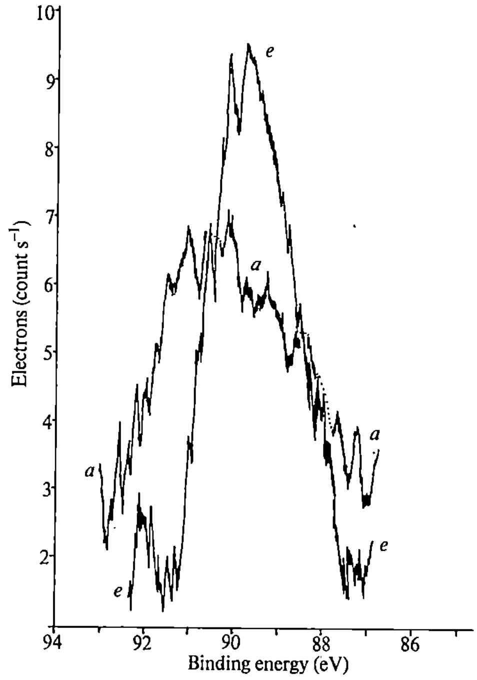

Fig. 3 X-ray photoelectron peaks for magnesium 2s electrons in the cell wall of *Lactobacillus plantarum* (*a*) containing esterified D-alanine and (*b*) with D-alanine removed.

The most significant result is the wide peak for Mg 2s electrons for both *B. licheniformis* (Fig. 2) and *L. plantarum* walls (Fig. 3*a*). This peak may be deconvoluted into two peaks on the basis of the measured width of the Mg 2s peak in magnesium phosphate. When the alanyl residues are removed from the *L. plantarum* samples there is a dramatic reduction of width to give the normal width expected for Mg 2s electrons (Fig. 3*e*). The measured binding energies thus indicate that the amino acid influences the ionic interactions of some of the magnesium ions. The interactions may be varied and complex, and the spectra may be properly interpreted in terms of many closely overlapping peaks rather than the two peaks shown. What is clear is that some of the magnesium ions show a higher 2s electron-binding energy when the amino acid is present than when it is absent. The nitrogen binding energy in both cell walls comes in the region expected for an NH_2 group[9].

Although the intensities in the X-ray photoelectron spectrum will be affected by differences in surface structure and sample state, in the case of the *L. plantarum* wall with and without the amino acids such differences may be small. The number of atoms of bound Mg^{2+}, estimated from the area under the Mg 2s peaks in Fig. 3, was reduced by 12% when the amino acids were present. This compares with a reduction of 25% measured by atomic absorption spectroscopy. Deconvolution of peak A in Fig. 3 into two component peaks indicated that 58% of the Mg^{2+} had the higher Mg 2s binding energy (90.6 eV) and 42% had the lower (89.6 eV).

Because, as we have already described, a higher Mg^{2+} electron binding energy represents a weaker ionic interaction between the Mg^{2+} and anions in the sample, the results indicate that in the presence of esterified alanine residues, a fraction of the Mg^{2+} is less strongly bound to the wall than that in the alanine-free walls, where it is known that the ionic interaction is dependent on phosphate groups[4]. The 2s electron binding energy in the weakly-bound Mg^{2+} corresponds to that in magnesium chloride, while the electron binding energy of the strongly-bound Mg^{2+} correlates with that of magnesium ions in magnesium hydrogen phosphate, in which the Mg^{2+} interacts with two P–O^- groups. We suggest that the strongly bound magnesium ions interact with the wall teichoic acid as indicated in Fig. 5*a*, but that in the presence of alanine the $-NH_3{}^+$ of the amino acid may neutralize the charge on an adjacent phosphate group, thus permitting only one of the pair of phosphates to interact with Mg^{2+} as shown in Fig. 5*b*.

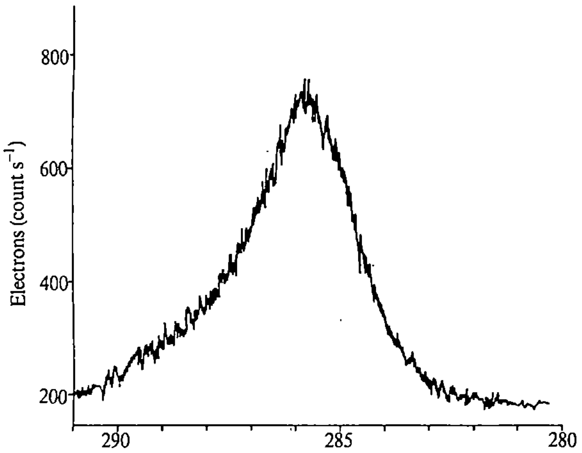

Fig. 4 X-ray photoelectron peak for carbon 1s electrons in the cell wall of *Bacillus licheniformis*.

A consequence of this model is that in alanylated teichoic acid the weakly bound fraction of the magnesium ions might compete less successfully with other cations for the anionic sites on the polymer and the preparation is likely to contain monovalent cations as well as Mg^{2+}. This would account for the non-stoichiometric reduction of Mg^{2+} binding by walls which contain alanyl teichoic acid[4,10] and which can be seen in Table 1.

It is possible that because of the flexibility of the polymer chain and the freedom of movement of the alanyl group a given alanyl residue may be capable of neutralizing one of a number of phosphate groups. A dynamic state in which the amino group alternates between several phosphates would produce changes in the positions on the polymer chain at which Mg^{2+} could bind strongly to two phosphates, and could thus produce a net movement of strongly bound Mg^{2+} on the polymer chain. This behaviour would be consistent with the known function of teichoic acids in both walls and membranes in producing and maintaining an appropriate divalent cationic environment[6].

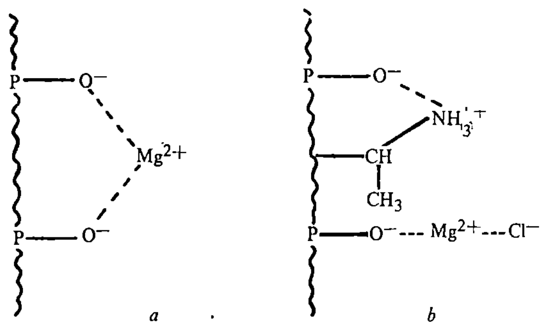

Fig. 5 Interaction of magnesium ions with wall teichoic acid.

The carbon 1s electrons reveal other structural features of the cell wall. Thus this region for *B. licheniformis* (Fig. 4) shows an intense peak due to C–H carbons and pump oil carbon, together with peaks at higher binding energy due to the various C–O and C=O groups.

We thank Dr Julia Douglas for cell walls of *Lactobacillus plantarum*.

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The Brassins

A SERIES of papers[1–4] has described experiments purporting to show the existence of brassins from pollen of rape (*Brassica napus* Gaertn). It is claimed that they represent a novel group[1]; that they consist of five active components; that they have a fatty nature[1,2]; and that they have plant hormonal activity[1–4]. We consider that the evidence presented in the four papers is inadequate to support any of these conclusions.

Our major criticism is that all the results reported were obtained with a mixture. No attempt was made to correlate quantitative measurement of any physical property with the intensity of a biological response; consequently there is no reason to relate any physical determination with the active molecule(s). No two measurements need necessarily relate to the same component of the mixture. Even the simple and fundamental separation of the extract into ether-soluble acidic, basic and neutral compounds was not attempted.

To determine the constitution of the brassins a fraction was separated[1] from an ether extract of dry rape pollen by TLC of the crude extract on silica gel, developing with benzene–methanol–acetic acid (45 : 8 : 4); the active fraction (R_F 0.35 to 0.45) was then rechromatographed with *iso*propyl ether–acetic acid (19 : 1). The activity remained on the origin and the eluate was termed brassins.

The crude brassin fraction was subjected to NMR analysis in $CDCl_3$ solution[1]. No integration or multiplicity of observed resonances was given and yet from a few undefined signals in the δ 1.26 to 2.00 region, a "signal" at δ 3.8 ("CH_2 attached to oxygen"), and a "multiplicity character" at δ 5.3 ("olefinic protons") the authors decide that this crude fraction is fatty acid ester material and suggest a glyceride structure. This information is insufficient to warrant these structural conclusions. The active substance(s) may have been so dilute that it (they) escaped detection by NMR spectroscopy. In the NMR spectrum of tristearin[5] in $CDCl_3$ the glyceryl methylene and methine protons comprise an A_2B_2C system with resonances centred around δ 4.2 and δ 5.3 respectively. No saponification of these "fatty acid esters" was attempted to support the authors' structural hypotheses.

The brassin fraction was not tested in any of the familiar bioassays which are reasonably specific for auxins (oat coleoptile elongation), gibberellins (growth stimulation of dwarf maize or α-amylase synthesis in barley aleurone) and cytokinins (tobacco pith callus growth), therefore a contribution of these hormones to the activity of the brassin fraction has not been excluded.

Combined GLC-MS of the crude brassin fraction was carried out and "several components" were observed and some features of their mass spectra were recorded[1]. No attempt was made to correlate the biological activity with observed GLC peaks and no fractions were collected and tested. Apparently no derivatives were prepared (for example, by methylation or trimethylsilylation of acidic components) before GLC to render volatile any involatile and possibly active components and so several may have escaped detection.

'Sephadex LH-20' gel chromatography, monitored by ultraviolet absorption at 254 nm, gave five peaks, all containing active material; but the conclusion that five active substances were present is not justified. These five ultraviolet absorbing fractions could overlie a spread of one active material—it is necessary to demonstrate inactive areas between peaks of biological activity before the presence of more than one active component can be claimed; even then the possibility of an inhibitor would have to be excluded.

The brassins were originally extracted from rape pollen but no experiments have been carried out to determine their effect on the physiology of rape pollen, or even on a rape plant. Consequently there is no direct evidence for their having a hormonal role *in vivo*. The extract of rape pollen undoubtedly accelerates the growth of bean (*Phaseolus vulgaris*) test plants, but the activity may be mainly attributable to a gibberellin because the elongation of internodes is a characteristic gibberellin response. Gibberellin activity occurs in high concentrations in ripe anthers (4 μg GA_3 equivalents g^{-1} dry weight in *Vitis*)[6]. The dual cell division and cell elongation response of bean internodes to brassins was reported to be histologically different from that induced by GA_3, because GA_3 did not induce cell division[3]. GA_3, has, however, been reported to stimulate cell division and elongation in petioles of strawberry[7], stems of *Hyoscyamus*[8] and even of internodal cells of bean (*P. vulgaris*, c. var. Blue Lake)[9,10], so the claim of "a new kind of growth effect"[3] is unfounded. The greater responsiveness of small (slow-growing) bean plants to applications of brassins is similar to the more pronounced effects of gibberellins on dwarf plants[11]; furthermore, the presence of more open flowers on brassin-treated bean plants[4] than on controls is also a typical response to gibberellins (see illustration in refs. 12, 13 or 14). In the light of the foregoing comments, and those of Stowe and Dott[15], it is idle to criticize the postulated roles of the brassins, concepts such as "alpha hormone"[4] and the suggestion that "... brassins seem to function early in the development of the embryo, . . . "; suffice it to say that the only data reported from experiments which involved embryos were the weights of ripe bean seeds produced on plants treated with brassins. We consider that the claims advanced for brassins being a novel complex of fatty plant hormones are premature and unjustified on the basis of work published so far.

Note added in proof. Since this manuscript was completed Mandava and Mitchell[16] have published a further paper on the "brassins".

They conclude from NMR and mass spectroscopy data of derivatives that "brassins" are glucose esters of fatty acids; this conclusion is inconsistent with NMR data given and earlier NMR and UV data[1] cited as evidence for a glyceride structure. The authors have not correlated biological activity with any physical measurement, consequently the attribution of "brassin" activity to any specific substance is unjustified.

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BOOK REVIEWS

Mythical Quagmire

From Honey to Ashes: an Introduction to a Science of Mythology. By Claude Lévi-Strauss. Translated from the French by John and Doreen Weightman. Pp. 512. (Jonathan Cape: London, February 1973.) £6.50.

THIS is the translation of *Du Miel aux Cendres,* the second volume in Claude Lévi-Strauss's magnum opus, *Les Mythologiques.* We are promised English editions of the third and fourth volumes in due course, and we can only admire the stamina of the translators in tackling this particular *Hydra.* At the same time, however, we might wonder whether, when the monster is finished, it will have been worth the effort.

The first volume in this *Science of Mythology* (as the term *Mythologique* has been unfortunately translated), *The Raw and the Cooked* (reviewed *Nature,* **226**, 875; 1970), is concerned with the exploration of the opposition of the empirical categories raw and cooked food as they are expressed in South American Indian mythology. This second volume continues this examination of the use of culinary categories although this fact is less easily concluded from the title. Here the opposition is between honey and tobacco, which, according to Lévi-Strauss, can be stated in the following culinary terms: honey comes from nature raw and ready for consumption and because it needs no preparation it belongs to the "hither side" of cooking, it is infra-culinary. On the other hand, tobacco is consumed neither raw nor cooked but its consumption involves reducing it to ashes and thus belongs "beyond" cooking, it is super-culinary. This initial opposition later requires modification because it only represents the natural properties of the two substances and their characters may be changed by cultural processes such as the fermentation of honey or the extraction of tobacco juice. Once again the material in which these oppositions and their meanings are explored is South American mythology; a large proportion of the 187 myths used in the first volume are re-examined together with 166 new ones.

It is virtually impossible to give any coherent picture of the content of this book, and for the purpose of this review it will suffice to note the aim and the conclusion. The aim is nothing less than to reveal the architecture of the mind as it is represented by mythic thought. The author claims that in this volume he has made an important step in that direction because his analyses have shown that the myths contain not simply a logic of qualities (the oppositions of raw/cooked, wet/dry, and so on) but also a logic of forms (the oppositions of empty/full, internal/external, and so on). He argues that the analysis has reached the point "where mythic thought transcends itself and, going beyond images retaining some relationship with concrete experience, operates in a world of concepts which have been released from any such obligation, and combine with each other in free association". This is the point, it is claimed, at which mythology gives way to philosophy and the existence of the latter is the necessary precondition of scientific thought. In other words, latent in mythic thought is scientific thought awaiting the right time and conditions to emerge. The unity of the human mind is assured.

What is the general reader to make of it? Probably not very much. He is assured that there is no need for him to read the first volume in order to understand the second because "the world of mythology is round . . . the reader can begin where he chooses and yet be sure of completing the course". While one must take the author's view on this characteristic of his work, it is a little difficult to see how the reader new to these works could understand what is going on without reading at least the overture to *The Raw and the Cooked.* (In fact, in the foreword to the third volume it is admitted that the world of mythology is not quite as round as had been assumed.) A second difficulty is the weight of ethnographic material which the reader is asked to carry in his mind. The load has become insupportable, and although one must admire the author for achieving the control he has of such a mass of evidence (zoological, botanical, and cosmological as well as ethnographical) it is only too easy to fault him. Some of his admirers claim that such criticisms are small-minded and unimportant compared with the grandeur of his aims. While the specialized reader may be dismayed by Lévi-Strauss's cavalier attitude towards and interpretation of the ethnography, however, he cannot fail to be impressed, perhaps even excited, by the quite extraordinarily imaginative associations and connexions which he makes. For those who are sceptical about these, it might be pointed out that recent field research in South America has demonstrated that these sorts of oppositions and analogies actually exist in the consciousness of Amerindians who seem to elaborate them as an intellectual exercise. This raises the further questions whether it would not have been easier for Lévi-Strauss to have achieved the same result more reliably through intensive fieldwork, or whether it has only been as a result of his intuitions that this mythological thought has been discovered to exist.

But a more serious difficulty for any reader does not lie simply in the mass of material, but in the nature of the analytical framework. It might be hoped that after over 800 pages of analyses some analytical tools and concepts would have taken on some more rigorous nature. There is no sign that Lévi-Strauss has made any attempt to produce such definitions, and the analytical procedures remain as vague and pliable as they are at the beginning of volume 1. In fact, the situation is worse because suddenly on page 249 he reintroduces an algebraic formula (of doubtful logical validity), first seen in his *The Structural Study of Myth* (1955) and not since, and declares that it has never ceased to guide him. The central notions of transformation and opposition still lack definition, terms such as armature and code still fail to convey any sure sense, and the result is that the reader, lacking any certain methodological path, tends to lose his way in the quagmire of myths. The work is studded with claims that this has been demonstrated, that has been proved, but the conclusions that are drawn do not seem necessarily to arise from the analyses. In the end there is just frustration.

PETER RIVIÈRE

Psychological Approaches

Humanistic Psychology and the Research Tradition: Their Several Virtues. By I. L. Child. Pp. vi+213. (John Wiley: New York and London, January 1973.) £4.15.

PROFESSOR CHILD was the author of *Italian or American*, a classic study of the conflicts created in children by discrepancy between the values and style of life of their home and those of the outside society. Although now 30 years old, this study should still demand for its author a respectful reading of any fresh work. The present book, moreover, is particularly sympathetic in approach: it attempts to reconcile different attitudes and points of view about human beings, to extract constructively the best of each, and to reconcile rather than oppose. By "humanistic psychology", Professor Child means those currents of thought in which man is viewed somewhat as he normally sees himself—as a person rather than only as an animal or a machine. The "research tradition", on the other hand, is that of systematic factual enquiry, in which the ideas and methods of study may be highly impersonal in their pursuit of objectivity. The technique of reconciliation which the book uses is to review researches conducted in the empirical tradition, but whose results and interpretation would favour the humanistic attitude. Thus there are chapters on moral development, on the psychology of art, on hypnosis, on cognitive dissonance, on extrasensory perception (ESP), and on clinical work in schizophrenia, psychotherapy, and behaviour therapy. In each of these areas, the choice of investigations is interesting, and they are very well described. Few of us can fail to be interested by evidence for the incidence of ESP being greater in people who believe in it, by agreement between art experts from very different societies on the relative merits of different artists; or by an apparent regression of ethical judgment in some people of university age as they work through to a higher level of adjustment.

Professor Child's praise and criticism of each approach, furthermore, are sound and well judged. In claiming that their virtues might be combined, and their vices abandoned, he is on very sound ground. The book ought therefore to be consulted by anybody who would like to achieve this end.

And yet, this smooth reconciliation does conceal genuine abrasive disagreements, which an upholder of the research tradition must emphasize. In several places, the humanistic conclusion from one study contradicts that from another. Moral development is supposed to be independent of the society in which it occurs: and yet the differences in behaviour in prisons, mental hospitals, and everyday life are to be ascribed to the different environments rather than to the individual. The best judges of artistic merit are those who reject the social pressure of others around them: and yet certain techniques derived from the Esalen Institute are suggested to increase hypnotizability, and this is regarded as desirable. A finding that those schizophrenics who report themselves as highly disturbed in a questionnaire also do very badly by objective tests of performance is interpreted as meaning that both scores are the common result of faking to secure a stay in a congenial hospital environment. This interpretation seems highly questionable, and it is not clear that it is more humane than believing what the patient says. Lastly, this book deals only with conclusions about personal choice and self control of which the humanistic thinker may approve: a true reconciliation of the two approaches will need also to take into account the massive evidence which the research tradition has provided of the distortions, limitations, and constraints which the machinery of ourselves and our society may place upon that choice. The commonsense concept of personality may be true enough as far as it goes; and yet it is a problem rather than a solution.

DONALD E. BROADBENT

Machine Botany

A Computer-Mapped Flora: A Study of the County of Warwickshire. With a section on Bryophytes by T. Laflin, and contributed chapters by F. W. Shotton, H. Thorpe and G. T. Warwick. By D. A. Cadbury, J. G. Hawkes and R. C. Readett. Pp. ix+768. (Academic: London and New York, December 1971.) £10; $30.

IN 1949 it was decided that, as so many changes had taken place in habitats and plant distribution in the county since the publication of Bagnall's *Flora of Warwickshire* (1891), a new survey was needed. A single kilometre square selected at random from each block of four (tetrad) was adopted as the basis for detailed study, and recorders listed habitats and frequencies of species on standard recording forms. This information was eventually conveyed to punched tape for processing by a computer. An incremental graph plotter and a line printer produced maps of vascular plants and bryophytes.

The maps occupy 420 pages and those for vascular plants are of two kinds. The first, covering 718 species, are complex and give three main pieces of information: presence or absence of the particular species; the major habitats in which the species was recorded (by the use of nine different superimposed symbols); and an approximate idea of frequency of the species in each habitat. The second, covering fifty-nine less common species, are simpler, and indicate only presence and frequency. Two frequency categories—abundant to frequent, and occasional to rare—are distinguished in the maps, but unfortunately the authors give no quantitative definitions of these terms, so it is left to the reader's imagination (as it was probably left to the ingeniousness of the recorders) to decide what they mean. The distributions of 184 different bryophytes in a fruiting or non-fruiting condition are also mapped. A pocket at the end of the book contains twelve overlay maps which show, besides physical and meteorological features, woods, parklands, heaths and commons.

The check lists accompanying the maps include information on first Warwickshire records, habitats, and occurrence in neighbouring counties. There are keys to the local species of *Rubus fruticosus* agg., *Salix* (including hybrids), *Arctium*, *Hieracium*, and *Bromus*. Chapters on the physical background, geology and soils (with an excellent map showing soil types), historical geography (explaining the changing patterns of natural vegetation in the county from prehistoric times to the present day), and habitat studies, complete the flora.

A shortcoming of the work is the recording in but one square kilometre out of every four. Although it ensures an even survey the result is that only one-quarter of the county is covered, and in spite of the inclusion of records from some squares chosen non-randomly, about 65 per cent of the county is left unworked. Is this the reason why so many species known to the old Warwickshire botanists are "not recorded in the present survey"? How much more satisfying if all squares had been surveyed: but to have done this with the thoroughness of the present work would have been an impossible task. So we should consider *A Computer-Mapped Flora* as an academic exercise expertly planned and accomplished, rather than as a definitive county flora. It is the first flora in which habitat data have been mapped by a computer, and we are grateful to the authors for introducing us to an entirely new approach to vegetational research.

A. R. PERRY

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A New Curriculum for the Schools?

ONE of the principal preoccupations in British education is with the interface between secondary schools and later life, and particularly that between schools and the universities. The schools (or many of them) have traditionally thought of the sixth form, occupied chiefly by young people aged between sixteen and nineteen, as the limbo in which brighter students prepare to compete for the increasingly cut-throat competition for a place at a university, and in which lesser lights acquire the qualifications in the A-level examinations needed for some vocations, teaching for example. The result is that the sixth form is pedagogically both the best and the worst of British school education. It is a source of wonder that, in some schools, a handful of dedicated teachers can provide their star students with a large part of the curriculum they are likely to encounter when eventually they find their way to a university. But the sixth form curriculum is also a narrowing experience for these same students, who find themselves forced to specialize before their general education is complete. In the past few years, the stability of this system has in any case been undermined by the increasingly catholic interests of the students staying on into the sixth form, increasing numbers of whom are more concerned with vocational training of various kinds than with the academic rat-race.

This is why the Schools Council, chiefly responsible in Britain for innovation in the school curriculum, and the Standing Conference in University Entrance, the universities' watchdog of what are called "standards", have been labouring for the past four years on a formula to regulate the sixth form curriculum in such a manner as to cater for the increasingly diverse interests of students in the schools and the persistently rigorous expectations of university teachers, modified only to a small extent by their suspicion that it cannot be good, in some sense of that word, that all students over sixteen should be put through a specialized curriculum in order that the universities themselves should be relieved of what is ultimately their inescapable responsibility of deciding which students to accept. The outcome is a report from the Schools Council's Second Sixth Form Working Party under Dr E. W. H. Briault, and a companion document from the Joint Sixth Form Working Party under Dr C. C. Butler (Schools Council Working Papers 46 and 47). Mercifully, both documents say the same thing in different words. They agree that there should be a sixth form curriculum which entails that students completing five years of high school should follow a course made up of five distinct topics of study, and that those with academic inclinations should thereafter follow two of these topics in greater detail. Implicitly, the Schools Council committee report is a promise that secondary school teachers in Britain will accept this formula. Dr Butler's committee, by endorsing the same formula in many more words, implies a similar promise by the universities. It remains to be seen whether these promises can be met, but there is to be a period, no doubt lengthy, of feasibility trials which will allow both partners to the present muddle about secondary education to declare themselves.

The first thing to be said is that the new proposals are an improvement. The idea that all entrants to the sixth form should follow a course of studies which has five topics and not three will, if found acceptable by the schools, be a step towards a liberal education. Unhappily, the new arrangements will not necessarily mean that those who wish in due course to become specialists of one kind or another will be required, during this initial period, to read widely, which implies that schools will in future, as in the past, be able to train specialists if they wish. Similarly, dedicated teachers and single-minded students will, if they choose, be able to sacrifice general education on the altar of supposed profundity. And so far as customers are concerned, there remains the danger that the new apparatus of school examinations will be used by the universities not merely as a means of telling which are the potential academics among school leavers but also, as in the past, as a device for selecting as university students only those who have already demonstrated that they can survive a university course as pastiched at school. In other words, quite apart from the objection that the new system will not specifically forbid the degree of specialization at school which has been traditional, there is a danger that it will more conspicuously drive a wedge, at the beginning of the sixth form, between those with academic inclinations and those with more general interests. Only the most fierce commitment to the cause of general education, in the schools as well as in the universities, will ensure that events take a different course. It is to be hoped that those with an interest in the feasibility trials are not exclusively those who seek to use the new system as a way of implementing courses of specialized study comparable in most ways with the system of the past several decades. The danger is that by a kind of pedagogical Gresham's Law, the bad currency of pointless, premature and unnecessary specialization will drive out the currencies of education in the round.

The second thing to say is that the new proposals, however they may be an improvement on present arrangements, are but an awkward device for avoiding several more fundamental questions about the relationship between school and university education in Britain. Both reports say explicitly that it is for universities to decide how students should be selected, but the British system is already shot through with the tradition that students should be selected for academic work in specialist departments at universities on the basis of their showing in similar studies at school. Is it not high time that British universities followed other university systems in asking whether the criterion of choice should be some more general test of a young person's academic potential? After all, less formally structured educational systems like the American, and more formally structured systems like the French, manage to base the selection of university students on a measure of fitness in general education.

Another unspoken and unanswered question is whether it is proper that the transition between school and university in Britain should take place, as at present, at eighteen or nineteen. Might it not be more economical as well as wiser and more suited to the needs of young people if what is called the sixth form were for most intending university students an experience lasting one or two years, not two or three, and if university courses were simultaneously lengthened from three to four? Most probably the answer would be that Mrs Margaret Thatcher, like her predecessors and probably her successors, would not sanction such a change. But there is no objective reason to think it would necessarily be more expensive while, educationally, it would almost certainly be more prudent. It may be that the best is the enemy of the good, which is why it may be wise that British education should now settle for the formula which the Schools Council and the Standing Conference on University Entrance have devised. But there is very little doubt that the proposed arrangements will pre-empt a more radical change for the best part of a decade.

France Should Not Test

THE government of France is no doubt righteously indignant that its plans for testing nuclear weapons in the Pacific have run into fierce opposition, not merely from the governments of New Zealand and Australia but from French citizens as well. For, the government of France may ask, did not the Soviet Union, the United States and the United Kingdom take advantage of testing in the atmosphere to develop their existing armouries of nuclear weapons? Does not the People's Republic of China still assume the right not to comply with the terms of the Partial Test-Ban Treaty, now nearly a decade old? What cause is there for asking that France should voluntarily forgo privileges which other nations have assumed.

The simple truth is that the testing of nuclear weapons in the atmosphere, which dates back to 1945, is a dangerous procedure whose dangers were not recognized until the mid-1950s and not acknowledged by the governments concerned until the early 1960s. But there is no doubt that if the military testing programmes of the superpowers had been begun at a time when the hazards of radioactive fallout were as widely understood as they are today, the protests would have been much more vociferous and the programmes more quickly brought to a halt. In retrospect, the existing nuclear powers may cynically be said to have been lucky that they began to develop their weapons at a time when ignorance of the consequences of radioactive fallout was widespread. (It is also, of course, fair to say that both the United States and the British governments of the 1950s did reprehensibly do much to minimize or even conceal the dangers of fallout, while the Soviet government has never openly admitted that these dangers are real.) But none of this can justify the way in which, a decade after the Partial Test-Ban Treaty, the French government now pretends that it too should enjoy the military convenience of a state of ignorance long since dispelled. The truth is that even if the series of tests now proposed by the French is numerically modest and even if the total yield of the explosions is comparatively small, there is nevertheless no doubt that the testing programme will add to the reservoir of radioactive material in the atmosphere. Since there is every reason to think that many of the consequences of fallout are linearly related to the dose, however small it may be, the governments of all countries, not merely the governments of those in the immediate vicinity, have a right to protest at what the French government now plans.

So how should the government of France respond? The simplest and most honourable course would be to abandon the testing programme. On the face of things, such an act would also imply to outsiders that the French government's *force de frappe* was less of a strategic reality than Mr Michel Debré has been saying. In reality, however, the French government would be well advised to admit that the *force de frappe* is no more than an illusion and that its best course would be to use the protests at the proposed testing programme in the Pacific as an excuse not merely for abandoning the tests but also as a starting point for serious discussions, within the framework of the European Community, about long-term strategic defence.

What about China? The Chinese have of course been testing nuclear weapons for the best part of a decade, but only in the past year have they entered into meaningful diplomatic relationships with the rest of the world and the United Nations in particular. The first need is to make sure that the Chinese in their new role are prepared to follow the rules of diplomacy, such as they are ; the experience of the past few months in the United Nations and of the bilateral negotiations with Japan and Western powers in particular have suggested that the Chinese are prepared to toe the line. So is there any reason why they should not now be asked also to comply with the spirit of the Partial Test-Ban Treaty and to abandon their own tests? The French would be on weaker ground if it were clear that the Chinese were also under pressure.

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100 Years Ago

NATURE

A GENTLEMAN writes us that he was invited by the Royal Commissioners to act as a juror at the Vienna Exhibition, but was at the same time coolly told that our Philistian Government had placed no funds at the disposal of the Commissioners wherewith to defray the necessary expenses of those who are willing to devote their valuable time and experience to the service of their country. Our readers will not be surprised at this. Other Governments have discovered that even in the most commercial, as well as in the highest light, the encouragement of science "pays." The British Government, with five millions on the right side of their account, still regard science as a beggarly Lazarus, to whom, for mere shame's sake, they are compelled to throw an occasional crumb. As our correspondent says, poor little Switzerland has devoted two and a half times the pittance our Government have allowed to defray the expenses of the Vienna Commission ; while the amount expended by Austria in their department of former exhibitions was at least four times as much as we have devoted to theirs.

From Nature, **8**, 54, *May* 15, 1873.

OLD WORLD

Fusion Research at Culham Expands

OUTLINE design studies on a fusion reactor are under way at the Culham laboratory of the United Kingdom Atomic Energy Authority. Work in the past five years has led to a much more optimistic assessment of the chances of fusion being used to provide electrical power, and support for the AEA's fusion work is to be increased from £3.9 million this year to £5 million a year by 1975–76. This reverses the decision taken in 1968 by the Labour Government and Mr Anthony Wedgwood Benn to halve the support for fusion research.

The decision to increase support follows the report by Sir Harrie Massey to Mr Tom Boardman, Minister for Industry, in which the AEA's fusion work was examined.

Dr R. S. Pease, Director of Culham, said last week that developments in high temperature plasma physics, in the understanding of toroidal magnetic field systems, particularly toroidal pinches, and in methods of suppressing the rapid diffusion (Bohm diffusion) that leads to poor thermal insulation, have all made the practical development of a fusion reactor a distinct possibility.

The enlarged programme has also been made possible by cooperation with Euratom. The—at any rate partial—solution to Euratom's future that was agreed on February 5 included an increased fusion research programme. Although this decision has still to be ratified, the Culham fusion programme will be jointly managed by the UKAEA and Euratom as part of a Euratom programme that will be carried out chiefly in national laboratories and which will be running at between £20 million and £25 million a year. Research at Culham will constitute about a quarter of the total, Dr Pease said, and about £1.25 million of the money spent at Culham will come from Euratom. The remainder will be provided by the UKAEA.

Dr Pease was also at pains to stress the importance of cooperation with the United States and the Soviet Union, and he cited the studies carried out in 1969 with the Soviet Union on Tokamak T-3 which has provided some of the best results yet with these experimental fusion assemblies.

The eventual aim of the enlarged programme at Culham will be to produce a plasma with a temperature of 100 million K and a density of 10^{14} cm^{-3} with a confinement time of over a second. Culham will try and reach this goal with continued effort on confining magnetic fields within toroidal magnets. At present Culham has three new experimental assemblies in operation in order to achieve this goal. First, there is a superconducting levatron; second, a high beta toroidal experiment (HBTX); and third, a CLEO Tokamak/stellerator experiment. The laboratory also plans to build further stellerator and Tokamak apparatus in the near future.

Each one of these pieces of equipment enables different facets of fusion to be examined. In the Levitron, for example, long confinement times can be studied—times of up to 1/10 sec have been obtained with this apparatus but the density of the plasma has been low. In the HBTX there is a dense plasma but unfortunately a lower containment time, while in the CLEO Tokamak apparatus discharges lasting 1/10 sec have been obtained with poorly conducting walls (such as would be obtained in a reactor) but when a combination of optimum density and good temperatures is obtained the confinement time drops to 1/100 sec.

Dr R. S. Pease

The next step according to Dr Pease is the construction of a large apparatus in which a high density, high temperatures and a long containment time can be simultaneously maintained. The apparatus will be toroidal and probably a tokamak. Culham is at present actively discussing with Euratom where this apparatus should be constructed. But wherever that might be it is planned to have it in operation by 1978.

If the problems of maintaining a plasma at high temperatures and high density for long times can be beaten then the next step is to develop a fusion reactor that can produce electricity commercially. This is a long way ahead although Dr Pease feels that when the large apparatus is built there will be a chance, however slight, that fusion power will be a reality before the end of the century. It remains to be seen whether this is more of a hope than an expectation.

EMBO

New Secretary

DR JOHN TOOZE is to be the Executive Secretary of the European Molecular Biology Organization. The decision, taken by the EMBO council last week, will mean that Dr Tooze will relinquish his position as research administrator at the Imperial Cancer Research Fund in London. Dr Tooze has been a regular contributor to *Nature* for the past seven years, and was deputy editor in 1968 and 1969.

Dr Tooze takes over the job from Dr Raymond Appleyard who is himself taking up another job with the European Commission in Brussels. Dr Appleyard, who was director and head of the biology programme in the Industrial, Technological and Scientific Affairs division, is now the Director General in the Dissemination of Information division.

The agreement among ten of the fourteen EMBO countries to build a European Molecular Biology laboratory in Heidelberg is to be signed later this week in Geneva. Before building can start the agreement will have to be ratified by the respective governments, a process which could take as long as a year. Although EMBO and the proposed laboratory are not directly linked, the new Executive Secretary will set up office in Heidelberg, but this will not happen for about 18 months. In the meantime EMBO will continue to be managed from Brussels.

CRIMOND TERMINAL

Loch Reprieved

TOTAL Oil Marine and the British Gas Corporation have put their plans to build a gas processing plant above the Loch of Strathbeg near Aberdeen into abeyance. The purchase of an alternative site at St Fergus, three and a half miles south of the loch, is being arranged and the plant may well be built there.

The change of plan became apparent when Mr F. E. Dean, the Gas Corporation's Chief Technical Liaison Officer, wrote to Aberdeen County Council informing it that the alternative planning application was being prepared. The chief reason for the change is said to be that the Ministry of Defence, which already has planning permission to build a radio station on Crimond airfield. above the loch, is still eager to use the site. A Gas Corporation spokesman added this week, however, that the protests from conservationists in the area who were worried that the plant might seriously damage the loch, which the Nature Conservancy classifies as being of grade one scientific importance (see *Nature*, **242**, 93 ; 1973), did have some effect.

The St Fergus site is much less valuable than the Loch of Strathbeg which is Britain's largest dune slack pool. The slack pools, dunes and bird-life at Blackwater, just north of St Fergus, where it is thought the plant will be sited, are far less likely to be disturbed by the station than at the Strathbeg site.

The Gas Corporation was at pains to point out this week that planning application for Crimond has merely gone into abeyance, and if the Blackwater site proves impossible, Crimond may still be the chosen spot. It is unlikely, however, that Total and the Gas Corporation would get the site without a planning inquiry if the Ministry of Defence seriously wants it. Work was due to start on the ministry's radio station this year, with completion planned for 1975, but the plan to put a gas processing plant on Crimond may have held work up.

BRITISH MUSEUM

Chaotic Jubilee

THE British Museum Research Laboratory is celebrating its Golden Jubilee with an exhibition which opened on May 4. At the entrance to the exhibition blowups of several historic press cuttings are displayed in a sepia coloured anteroom simulating the conditions under which the first workers in the laboratory laboured. Among these cuttings is one from the *Daily Herald* of December 31, 1931:

"I am certain that Dr Alexander Scott (FRS) could put Humpty Dumpty together again. That would be a small job for the head of the laboratory which cleans and restores the priceless exhibits of the British Museum."

So begins the cutting. And perhaps Dr Scott's sure touch would have helped in the preparation of this exhibition, for at the press showing on May 3 the display was still being put together, and seemed to be in about as many pieces as Humpty Dumpty after his fall. That makes it difficult to assess the merits of the exhibition, housed within a double dome inflated by air pressure, in the former ethnography gallery. Undoubtedly the show will be worth a visit in calmer conditions, when it will be possible to see if the rainbow coloured lighting and technological exhibitionism of the surroundings add to, or detract from, the exhibits, which will include the use of radiography, metallography, X-ray diffraction analysis, atomic absorption analysis and both carbon-14 and thermoluminescence dating.

The overall appeal of the exhibition seems likely to be visual rather than scientific. One already complete exhibit, for example, shows a glass vase which appears pea soup green in reflected light and magenta in transmitted light. This effect has been duplicated at the laboratory by adding sundry traces of metallic oxides to glass —but this seems very much window dressing compared with the real work of the laboratory.

It is, however, obvious that the staff of the laboratory has achieved much under difficult conditions. Not only scientific problems but bureaucratic difficulties have confronted them. In another cutting displayed at the show, quoting from the *Birmingham Post* sometime in 1932, we learn that "The laws and regulations under which museum authorities work are strict. As a precaution against fire and explosion there is no gas on the premises. Some years ago a room was set apart as a laboratory, and a supply of gas became essential, but the regulations could not be broken. The difficulty was overcome by carrying a pipe to the outside of the laboratory window, and then continuing the supply to the desired position by means of a rubber tube that could be disconnected every night".

Also, "No exhibit can be taken away for treatment. Once an exhibit has been accepted by the trustees an Act of Parliament would be necessary to permit its removal from the premises. The laboratory in Russell Square . . . is made technically part of the Museum by a connecting corridor across the grounds. Every article in need of treatment . . . has to be carried that way so that there shall be no direct breach of the rules." And seemingly it still requires an Act of Parliament to remove articles from the Museum, for example when exhibits are displayed abroad.

Although when the laboratory was first set up in 1922 *Nature* commented that "National laboratories for the cleaning, restoration, and preservation of antiquities have for some years existed at Berlin, Copenhagen and Stockholm" (**109**, 119; 1922), it is today the oldest museum laboratory in existence. So, having started perhaps somewhat ingloriously, primarily concerned in the first years with the restoration of objects which had been damaged when stored in a section of the Underground during the First World War, the staff have now thoroughly earned their right to a Jubilee junket.

PESTICIDES

Too Much Concern

CONCERN about the use of pesticides has sometimes been due to misinterpretation or improper extrapolation of scientific data, according to a World Health Organization expert committee on insecticides (*Safe Use of Pesticides*, Report No. 513, WHO, Geneva, 1973). This has caused some administrations to impose restrictions on the use of certain pesticides which cannot be justified on the basis of present scientific knowledge, says the committee.

The nub of the report is that the advantages of the use of pesticides, in particular DDT, in controlling diseases outweigh their disadvantages. The committee warns that the withdrawal of DDT from areas which in the past have been ravaged with malaria could well see the return of the disease.

But the committee accepts that there is insufficient evidence available on the effects of DDT on man. It recommends that comprehensive metabolic studies be carried out of the effect of the disease to complement studies on the carcinogenicity of the pesticide. The committee points out, however, that even though the first reports that DDT might cause tumours in rats appeared in 1947, there has been no adverse effect observed in a small group of men who have been exposed to heavy doses and who have been under survey for the past twenty years.

Education is the answer to the hysteria caused in recent years by the supposed effects of pesticides according to the committee. The report says that even in developed countries, people need to be introduced to the basic principles of toxicology "in order to dispel the excessive concern over hypothetical but unproven effects of pesticides and to ensure a more realistic appreciation of hazards".

DDT is not the only topic covered in the report and the committee also makes a plea for the introduction of less toxic mercury compounds than those at present used as seed dressings and fungicides. The alkyl mercury compounds, the committee points out, accumulate in the nervous system giving rise to irreversible damage. So, "in the long view, it may be unwise to use any mercury compounds and certain other heavy metal compounds as pesticides".

NEW WORLD

Functional Organization Makes Trouble

by our Washington Correspondent

A SWEEPING reorganization of the federal government's health services has been set in train by Caspar W. Weinberger, the new Secretary of Health, Education and Welfare (HEW). Tighter management control is the watchword, and although some reorganization of HEW's sprawling bureaucracy takes place nearly every year, this particular round of executive musical chairs is of more than passing interest to the scientific community. The chief losers in the shuffle are mental health programmes in general and the National Institute of Mental Health (NIMH) in particular.

The nub of the plan is that the Health Services and Mental Health Administration should be dismantled, and that all the health programmes of the federal government should be reorganized into five separate agencies, the heads of which will report directly to Dr Charles C. Edwards, the new Assistant Secretary for Health. NIMH is being transferred almost lock, stock and barrel back into the National Institutes of Health, the agency which housed it until 1967 when NIMH was separated from the other biomedical research institutes and promoted to bureau status.

The five health agencies to be established by the reorganization are as follows:

- The Food and Drug Administration, which will be unchanged.
- The Center for Disease Control in Atlanta, Georgia, which will also be essentially unchanged but which will take on the National Institute for Occupational Safety.
- The National Institutes of Health, which will gain NIMH, but lose the Bureau of Health Manpower Education.
- A new Health Services Administration, which will be concerned with health care delivery.
- A new Health Resources Administration, which will be concerned with health service data gathering and demonstration programmes, and which will also take over the Bureau of Health Manpower Education from NIH.

Harold O. Buzzell, former Deputy Manpower Administrator for the Department of Labor and a management consultant who once worked for Edwards's former employers, Booz, Allen and Hamilton, has been appointed to implement the reorganization and eventually to direct the Health Services Administration.

The objectives of the shuffle are clear: to reorganize the health bureaucracy along more functional lines and to put the Assistant Secretary of Health in firm control of the federal government's health programmes. In the past, the assistant secretary's lines of control have been rather obscure, and he has often been bypassed on crucial decisions.

What all this means for biomedical research and the National Institutes of Health is a matter of debate, but a clear indication of the way the wind is blowing was given by Mr Weinberger during a briefing for a few reporters before the plan was announced. He was quoted as saying that the reorganization will help to "redress the balance" in research funding. "There has been too much emphasis on investigator-initiated research and not enough on directed research", he is reported to have said, and suggested that more research funding should be spent on attacking specific diseases such as cancer, and less on basic research.

Concentration on research likely to produce results in the short term at the expense of longer-term studies has become the general trend in research spending across the board in the United States in the past few years, but if Mr Weinberger had intended to antagonize much of the biomedical community, he could hardly have chosen his words more carefully. Already the cancer plan, which has not yet been published, has been bitterly attacked for stifling scientific creativity, and budget cuts which have reduced general research support in every institute in NIH have been attacked for jeopardizing the long-term future of biomedical research in the United States.

In organizational terms, the reorgani-

MARS

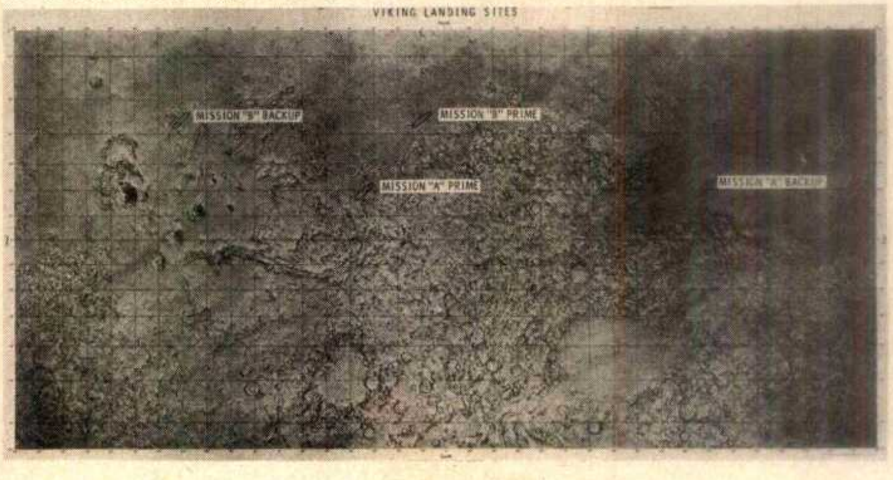
Targets for Viking

POSSIBLE landing sites for the Viking mission to Mars in 1976 have finally been chosen by NASA. After a year's intensive study of some 22 possible sites, NASA has chosen Chryse (19.5° N, 34° W) as the prime target for the first lander and Cydonia in the Mare Acidalium (44.3° N, 10° W), as the target for the second lander.

Chryse is situated at the northeast end of the 3,000-mile long, 20,000 feet deep rift valley discovered by Mariner 9, in an area which resembles a drainage basin, and it is expected to contain a variety of deposits of surface material. The first lander will visit the site in June 1976 if all goes well. The second landing site, which will receive another lander a month later, lies at the edge of the southernmost regions of the north polar hood — a hazy area which may carry moisture. Chryse is thus more interesting geologically, while Cydonia offers the best prospects of any life.

Both sites also lie well below the mean surface of the planet, which is an advantage in two respects. First, the denser atmosphere will provide more efficient braking for the parachutes, and second, the high atmospheric pressure adds to the chances of finding water. Backup sites for each lander are at the same latitudes as the prime sites.

A special advantage of the two prime sites is that both landers will be in communication range of both orbiters, which could enable one orbiter to be released from synchronous orbit for low-altitude study of other regions, while the second orbiter relays information back to Earth from both landers. The sites are tentative, and can be changed by retargeting the spacecraft up to 10 days before encounter.



zation plan has some interesting implications for NIH. First, after six years of independence, NIMH is being returned to the NIH fold. As far as NIMH's biomedical research is concerned, the transfer will probably not make much difference because it never really left the NIH campus, and there has continued to be considerable integration of facilities and programmes. Dr John Eberhart, Assistant NIMH Director for Mental Health Research, has continued to attend meetings of NIH programme directors, for example, and the NIH Division of Research Grants has provided many of the same services for NIMH that it provides for other institutes of NIH.

But the transfer does bring into the NIH many service activities which have not previously been its concern (although the cancer bill has recently given NIH authority to run cancer control programmes). Many such health services are connected with NIMH's drug abuse activities, which are being kept in the institute more because of legal difficulties than anything else. The Administration has been toying with the idea of either splitting them off into a separate addiction agency or transferring them to the Center for Disease Control, but because they were originally consigned to NIMH by act of Congress, they could only be split off with Congressional approval.

As for NIH's Bureau of Health Manpower Education, the Administration's proposed budget cuts and the non-renewal of legislation dealing with manpower for health and allied professions have severely reduced its activities and effectively separated its programmes from other NIH activities. Although the events which have led to such a situation have been painfully felt and bitterly attacked in the medical schools, they have removed much of the original justification for placing the bureau under NIH control.

The transfer of NIMH back to NIH has been called a downgrading of the federal government's mental health programmes. That may be so, but for NIMH the writing was on the wall in January, when President Nixon's budget was unveiled. The budget proposed phasing out federal funding of community mental health centres (CMHCs), a programme launched by President Kennedy in 1963 as a bold new approach to caring for the mentally ill. The CMHC programme has resulted in the setting up of about 400 centres providing mental health care on a community basis, funded partly by NIMH and partly by state and local governments. Ironically, the Administration has proposed that federal support should be withdrawn because the centres are so successful—the argument is that the states will pick up funding with money from general revenue sharing.

Sakharov Honoured

The National Academy of Sciences has elected Dr Andrei D. Sakharov, the dissident Soviet nuclear physicist, as a foreign associate. Sakharov, who played a leading role in development of the H-bomb in the Soviet Union and who has recently been in trouble with Soviet authorities for his championship of civil liberties, is one of only 12 scientists to be elected as foreign associates of the academy this year. Dr Philip Handler, NAS President, said last week that Sakharov's election was "by no means for political reasons"—his nomination came from the academy's physics section for original scientific research. He is, in fact, one of three Soviet scientists to be elected as foreign associates of the academy last week, the others being Vladimir Engelhardt, Institute of Molecular Biology, and I. S. Shlovsky, Institute of Space Research.

Other foreign associates elected were Sune Bergstrom, Karolinska Institutet, Stockholm; Albert Eschenmoser, Technische Hochschule, Zurich; Dennis Gabor, Imperial College, London; David Glass, London School of Economics; Jean Goguel, Ecole des Mines, Paris; Motoo Kimura, National Institute of Genetics, Japan; George Klein, Karolinska Institutet; Ben Roy Mottelson, Institute for Theoretical Physics, Copenhagen; and V. Ramalingaswami, All-India Institute of Medical Sciences, New Delhi.

NAS/NAE

Options Kept Open

by our Washington Correspondent

The annual meeting of the National Academy of Engineering last week passed a resolution requesting the NAE's governing council to prepare a plan for incorporating the academy as a non-profit corporation separate from the National Academy of Sciences. But several amendments to the resolution, proposed from the floor, have kept options open for the NAE and they should help to ensure that the two academies will be able to live in harmony whether or not their planned divorce is made final.

In short, the resolution authorizes (but does not direct) the council to seek approval at its annual meeting in October of its plan to establish the NAE as a corporation, but it also suggests that negotiations should be continued with the National Academy of Sciences, that a Congressional Charter should be prepared and that the members should be given at least two options for action before the next annual meeting. The chances are that the NAE will set up shop independently of the NAS, but NAE officials emphasized after the meeting that whatever the final outcome of the breakaway movement, they hope that the two academies will continue to work together on problems and studies of mutual interest.

The basis of the long-standing disagreement between the two academies is the management of the National Research Council, their joint operating arm. Governance of the NRC is vested solely in the hands of the Council of the NAS, and NAE officials want it to have a tighter management structure (see *Nature*, **243**, 5; 1973). The NAE, which was set up in 1964 and has since been operating under the charter of the NAS, also wants to be more visible in the engineering community.

Shockley Again

For the past seven years, Dr William Shockley, co-inventor of the transistor, has been trying to persuade the National Academy of Sciences to examine the evidence for his belief that genetic factors make blacks less intelligent than whites. He failed again last week when the annual meeting turned down his familiar resolution calling for a committee to study the matter, but this year the meeting passed a substitute resolution which may help to blunt criticism from Shockley's supporters that the academy is treating him like a latter-day Galileo.

Offered by the academy's governing council, the substitute resolution acknowledged Shockley's efforts to bring his studies to the academy's attention, and encouraged "him and others engaged in such efforts to follow the normal scientific procedures of publication so that their results may be subjected to the usual peer review and accorded the scientific impact afforded by such publication". A preamble to the resolution said that Shockley's previous attempts had been voted down without debate because "only a small minority of the membership has any expertise in the field and . . . many of those with expertise doubt that the field has progressed to the point at which a definitive conclusion is warranted".

NEWS AND VIEWS

Unravelling the Eukaryotic Chromosome

RECONCILING the visible structure of the eukaryotic chromosome with its molecular organization has for some time seemed to be an almost insuperable obstacle in preventing any real advance in understanding the control of gene activity at the molecular level. The chromosome has appeared more complex than the sum of its parts and the environment of the gene has remained virtually undefined. At the visible level, chromosomes contain two types of region—euchromatin, which has a diffuse structure and is active in the synthesis of RNA, and heterochromatin which appears less compact and is inactive in gene expression. Early attempts to use these two types of structure by finding differences between them which might throw some light on the structure of the chromosome all failed. But evidence that regions of the chromosome may be distinguished by their responses to certain dyes has been accumulating in recent months, and Comings and his colleagues now report in *Experimental Cell Research* (77, 469; 1973) that these experiments may reflect the uneven distribution along the chromosome of non-histone proteins and not, as had at first been thought, differences in sequences of DNA.

The first breakthrough in defining the structures of heterochromatin and euchromatin was provided by the demonstration that the heterochromatin at the centromeres of eukaryotic chromosomes is rich in sequences of satellite DNA, the component of the genome which consists of a short basic unit repeated many times in related but non-identical copies. The cytological hybridization used for these experiments showed that the centromeric heterochromatin is distinct in two respects; it hybridizes with satellite DNA sequences and it stains very deeply with Giemsa stain after the hybridization. When Arrhigi and Hsu and Yunis *et al.* showed that staining takes place in such heterochromatin whenever the chromosomes are treated with alkali and then incubated in a saline solution at 60° C for some hours—part of the protocol followed in cytological hybridization—it was thought that the unusual properties of the DNA might explain the deep staining.

An equally important discovery about chromosome structure within the euchromatin or the arms was the observation of Caspersson and his colleagues that treatment with quinacrines causes each chromosome of the human complement to fluoresce intensely in localized regions. In fact, the entire chromosome complement may be identified by the characteristic fluorescent banding patterns of each chromosome. A connexion between Giemsa staining and quinacrine fluorescence was then suggested by the work of Evans and his colleagues, who showed that the same banding patterns are produced by a very gentle treatment of the chromosomes with saline solution followed by staining with Giemsa. These techniques, which are now coming into widespread use, have been named C banding (alkaline treatment, incubation in saline solution at high temperature, Giemsa staining), Q banding (direct application of quinacrine) and G banding (incubation for 1 h in saline solution, staining with Giemsa).

An early hint that the preferential renaturation of repeated DNA sequences might be irrelevant to banding was provided by Stockert and Lisanti (*Chromosoma*, 37, 117; 1972), who found that the same C banding pattern is obtained no matter whether the DNA is single stranded or double stranded. By using the reagent acridine orange, which fluoresces orange with single strands of DNA and green with duplex strands, they demonstrated that the centromeric regions, as expected, renature the most rapidly after denaturation; but renaturation of all regions is complete within a few minutes. Comings *et al.* have now excluded another possible explanation—that the DNA is more concentrated within the centromeric heterochromatin and thus stains more deeply—by measuring the content of DNA along the chromosome with feulgen stain. The packing of DNA in centromeric heterochromatin seems if anything to be slightly less compact than in euchromatin. (They therefore suggest that the compression of DNA in heterochromatin may be relatively fixed and constant during the cell cycle; during interphase, when the euchromatin uncoils to a diffuse structure, heterochromatin therefore provides the most condensed regions but at mitosis when the euchromatin becomes compact also there is little difference between the structure of the two regions.)

The alkaline treatment which is used to denature DNA in the protocol for cytological hybridization seems to be the critical step in generating the intense C bands at the centromeres instead of the G bands along the chromosome arms. Comings *et al.* report that treatment with 0.07 M NaOH for 30 s removes 21% of the DNA from the chromosomes; and the incubation in saline overnight at 60° C which is the next step extracts a slightly greater amount. By the time the chromosomes are stained with Giemsa then, they have lost half of their total DNA; and the extracted sequences all derive from the arms rather than from the centromeres. The repetitive sequences of centromeric DNA must therefore be packaged in a structure much more resistant to extraction than that of the arms.

Is the preferential extraction of DNA sufficient to explain C banding? Because gentle treatment with trypsin or other proteolytic enzymes produces a G banding pattern in the arms, but more prolonged incubation abolishes the G bands and instead produces C bands, Comings *et al.* suggest that differences in the proteins associated with DNA may explain both C banding and G banding. Because extraction with acid does not produce a banding pattern, it seems likely that the non-histone proteins must be responsible for the definition of regions as bands or non-bands. According to this model, a gentle disruption of chromosome structure by short incubation in saline, treatment with trypsin and so forth, extracts proteins from the chromosome. Untreated chromosomes stain with Giemsa uniformly along the arms, but react less intensely at the centromeres. The extraction must therefore remove some proteins from the regions which cease to bind Giemsa, that is the non-bands, leaving Giemsa able to bind only to the bands retaining the proteins. More stringent treatment, either with saline at high temperature, or with trypsin, or with alkali, completely disrupts the structure of the arms by extracting either their proteins or DNA ; in either situation, the G bands and Q bands are abolished and the centromeres are converted in to a state in which they stain intensely instead of failing to react.

Three types of structure can therefore be defined in the chromosome by the banding techniques. Centromeres contain heterochromatin which is the least reactive region in G banding or Q banding but reacts intensely under the conditions of C banding. The arms of the chromosomes can be divided into bands which react with Giemsa and quinacrine and non-bands which fail to do so. One puzzle about the nature of the bands is that they react with quinacrine directly, but react with Giemsa only after pre-treatment has extracted some of the non-histone proteins, presumably from the non-banding regions. The similarity of the results produced by these two different treatments therefore argues that the bands may correspond to some important underlying structure of the chromosome and are distinct from the non-banding regions.

What is the nature of the material which forms bands? The centromeric heterochromatin contains sequences of highly repetitive DNA and in organisms where such sequences occupy extensive regions of chromosome, they too show C banding. This suggests that the repetitive satellite sequences differ in their chromosomal proteins from less repeated sequences ; this provides the first evidence to show that the distribution of proteins in the chromosome may be segregated according to the function of the different regions. But the properties of the bands in the arms is less well defined. It is tempting to speculate that they too form heterochromatin ; but this conclusion rests solely upon their staining properties—the traditional distinction of heterochromatin—because they have not yet been shown to have any of the other features characteristic of centromeric heterochromatin, such as genetic inactivity, late replication or repeated sequences of DNA. In fact, one of the most important conclusions drawn from the experiments of Comings *et al.* is that the evidence of previous experiments that repeated sequences are not present in the arms must be reconsidered; because these experiments used alkali, much of the DNA of the arms must have been extracted before the hybridization step, so that it gives little information about the sequences of the arms. The parallel between the C bands on the one hand, and the G bands and Q bands on the other, is therefore intriguing but incomplete ; whether or not the bands are heterochromatin, however, they represent sequences of structural distinction which if correlated with specific functions (or lack of functions) may begin to help to unravel the structure of the chromosome.

From our Molecular Genetics Correspondent

Block Copolymers

THE rationale of materials science as a discipline lies in the stimuli that the student of one category of materials derives from the study of a quite different category. Such stimuli most commonly derive from studies of microstructure and the experimental factors that control it. For a long time now, students of metals and of ceramics have learned from each other in this way. Until quite recently, polymers have been a family apart, but as progressively larger and more perfect polymer crystals have been grown, the whole metallurgical apparatus of crystal growth, grain structure and dislocation morphology has become increasingly relevant to polymers. Recent developments in the formation of block copolymers have now taken this slow rapprochement a stage further ; from now on the polymer scientist will need to concern himself with the microstructural disposition of two phases.

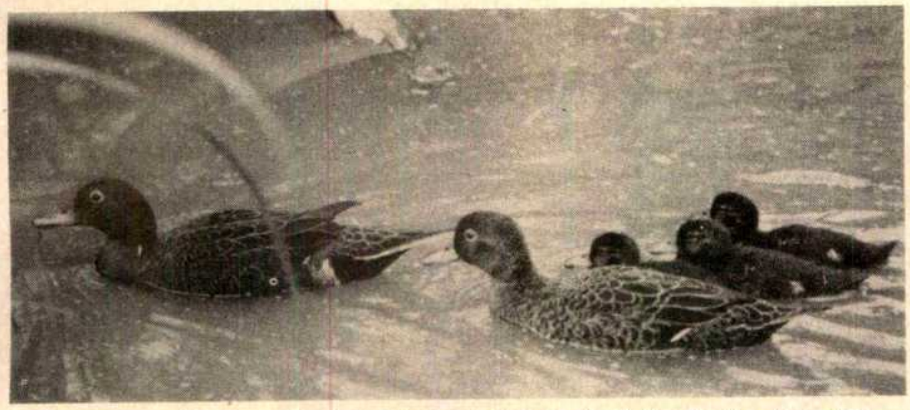
A block copolymer is ideally one in which a substantial block of one homopolymer is joined to one or more blocks of a chemically distinct homopolymer. A particularly important example is the rubbery (SBS) copolymer consisting of a polybutadiene block flanked by two polystyrene blocks (Holden, Bishop and Legge, *J. Polymer Sci., C*, **26**, 37 ; 1969). For these novel materials to develop their excellent mechanical properties, the individual blocks need to have closely controlled, uniform lengths—that is, both the total and the partial molecular weight must be fixed. This has become feasible through the technique of "living" polymerization, originated by Szwarc (*Nature*, **178**, 1168 ; 1956). This very important method depends on a special (anionic) method of maintaining the polymerization reaction so that no accidents can happen to "kill" the growing end of a polymer chain and thus lead to a broad spectrum of chain lengths. By ensuring rapid initiation and then controlling the growth of the population of chains by varying the species and concentration of monomer to which the population is successively exposed, block copolymers of rigorously controlled total and constituent molecular weights ensue.

Szwarc, who works at the State University of New York at Syracuse, has now published the first extensive review of the synthesis and properties of block and graft copolymers made by the living copolymer technique (*Polymer Eng. Sci.*, **13**, 1 ; 1973) ; every teacher of materials science should read it. Whether or not he is interested in the chemical details of synthesis, he is bound to be stimulated by the account of the microstructure of these polymers and their closely related properties. As the styrene/butadiene ratio is altered, two-block copolymers of these monomers show a sequence of structures closely reminiscent of metallic eutectics. The minority phase forms a regular array of spheres ; if its concentration is increased, the spheres give way to a hexagonal array of rods and these in turn to a lamellar morphology. Finally, matrix and disperse phase are inverted as the concentration of the original minority phase is further increased. One difference from metallic eutectics is the much finer scale ; polymer eutectics typically have a periodicity of only 10–20 nm. Another difference is that the phases are probably not to be regarded as true phases at all—Szwarc dubs them "mesophases"—because individual polymer chains are long enough to pass through several "interphase" boundaries.

Microstructures of SBS rubbers, which are similar, have been assiduously studied at Manchester University by Lewis and Price, for example, *Laboratory Practice*, **19**, 599 ; 1970). They produced their polymers in the form of electron-transparent films by solvent evaporation, and found that the scale of the pseudo-eutectic structure is modified by a change in the rate of evaporation. One is irresistibly reminded of the modification of metallic eutectic microstructures by a change in the freezing rate. Lewis

ZOOS

Breeding Waterfowl in Captivity



THE indispensable *International Zoo Yearbook*, (vol. 13, Zoological Society of London, 1973, £8) this year has a special section on waterfowl in captivity. The young of the rare New Zealand brown teal (*Anas aucklandica chlorotis*) shown here were hatched for the first time in captivity at the Wildfowl Trust, Slimbridge. Both parents tend the young, an unusual feature in dabbling ducks.

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and Price point out that the microstructure of a block copolymer has four levels of organization: primary chain structure; geometrical conformation of individual chains; microphase separation and disposition; and "grain structure". This last refers to the existence of regions in which the hexagonal array of polybutadiene microspheres lies on one "lattice", abutting on other regions or "grains" in which the lattice has a different orientation. Lewis and Price have even studied the arrangement of microspheres in the "grain boundaries", which are disturbed just like real grain boundaries in metals. It will certainly benefit the development of materials science that Lewis, a chemist, is now pursuing these studies in a metallurgy department.

Returning to Szwarc's review, a number of illustrations may be found of the advantages of a precise control of the chain structure of a block copolymer. (Note that the living polymer technique allows a polymer of a given gross composition to be produced in a variety of microstructures, corresponding to different block lengths, just as an alloy of fixed composition can be given different microstructures by varied heat-treatments.) The essential physical and chemical properties of the constituent blocks are preserved and not averaged out as in a random copolymer. Thus weak but permeable desalination membranes of cellulose acetate can be stiffened by making a block copolymer with polystyrene, which is more resistant to creep though not by itself permeable. Again, microstructures with dispersed arrays of rods can be stabilized by dissolving a block copolymer made by Szwarc's method in a suitable, chemically distinct monomer and then rapidly polymerizing the monomer. The rod structure, already existing in the solution, is thus fixed in a matrix which now incorporates the second polymer, and the whole is a form of ultrafine fibre-reinforced polymer, which can be aligned by extrusion.

Szwarc suggests a number of other technologically desirable properties which could be achieved by special forms of highly regular block copolymers, and calls for intense studies in this field. The call is fully justified.

From our Materials Science Correspondent

PROTEINS

Built to Last

from our Molecular Biology Correspondent

GIVEN the facts of hydrophobia in proteins, one would suppose that the lower limit of chain length to allow the formation of a stable globular conformation would be governed by the number of non-polar side chains needed to generate an "inside" from which water is excluded. So far the smallest proteins that can be categorically said to possess a unique, stable globular structure have fifty to sixty residues, and include the insulins ferredoxins, a family of venom neurotoxins and pancreatic trypsin inhibitor. This last is a remarkable specimen, for in spite of its small size, it exhibits a quite remarkable stability towards denaturants, such as guanidine hydrochloride, renatures from the reduced state with unusual speed and efficiency, and can be kept in solution without loss of activity literally for years. Its sequence and structure are known; it is without histidine or tryptophan, but has three disulphide bonds, providing a rather high cross-link density by the standards of larger proteins. Some remarkable aspects of its stability are now revealed in proton magnetic resonance studies from two laboratories.

Masson and Wüthrich (*FEBS Lett.*, **31**, 114; 1973) have concentrated their attention on the downfield region of the spectrum, where, below the phenylalanine and tyrosine ring resonances, an extensive series of peaks, mostly corresponding to single protons, and many of them resolved into doublets, are apparent in freshly prepared D_2O solutions. These are evidently refractory exchangeable hydrogens, presumably of internal peptide groups, showing coupling of the amide proton pairs. Guanidine hydrochloride makes no impression on these resonances until the solution is heated, when, at a sufficiently high temperature, they vanish. Renaturation by diluting out a solution of the protein in dimethyl sulphoxide with D_2O regenerates the NMR spectrum characteristic of the native state, except for the downfield peaks. Now resonances from shielded exchangeable protons have been observed in fresh solutions of a few other proteins, but they disappear quickly. In pancreatic trypsin inhibitor, on the other hand, of the fifteen observed features of this nature, four diminish noticeably in intensity on a time scale of hours, another six after several days, but the remainder appear to be indefinitely stable. With sufficient effort and ingenuity it may be possible to make specific assignments in terms of Huber's high-resolution X-ray structure. Slow exchange prevails also at *p*H 0.7, and even in alkali at *p*H 11 and higher, three of the shielded protons persist. Exchange in the native protein can be provoked by heating, and occurs in minutes at 65° C, whereas no unfolding can be detected by any of the usual methods until the temperature reaches 85° C.

Much the same results have been obtained by Karplus, Snyder and Sykes (*Biochemistry*, **12**, 1323; 1973), who waited for four months for the downfield resonances to go away, and at the end of this time the greater number were still there. The usual view of protein conformation is that it allows for a certain measure of undulation or "breathing". By the standards of other globular proteins that of pancreatic trypsin inhibitor is evidently quite remarkably resistant to any such fluctuations, except at high temperature; that is to say, there is an energetic barrier to any kind of unfolding of quite astronomical proportions.

In the alkaline range, the aromatic ring proton resonances of the tyrosine residues undergo shifts with the ionization of the phenolic groups. Thanks to the absence of other aromatic residues apart from phenylalanine, the spectrum in this region is relatively simple, and because the magnetic environments of the four tyrosine residues are all sensibly different, an ionization curve can be constructed for each, and the four peaks determined with good precision. Karplus *et al.* find, as did Masson and Wüthrich, that even in this alkaline range, some of the exchangeable peptide protons still remain unexchanged, and it seems that denaturation in these conditions is not wholly cooperative, and that even in so small a molecule independent cooperative units can remain tightly folded and resist the incursion of solvent.

Failure of cooperativity in denaturation, and the appearance of intermediates of long lifetime on the NMR time scale has now also been established for the archetypal case of ribonuclease. Thus Westmoreland and Matthews (*Proc. US Nat. Acad. Sci.*, **70**, 914; 1973), concentrating on the downfield histidine resonances—the separation of which because of the varied environments of these groups, is perhaps the most distinctive feature of the native spectrum—find that the acid denaturation profiles of the chemical shifts of these residues do not coincide. It is inferred that there are at least two separately unfolding regions in the structure, which is consistent with results of rapid kinetics of unfolding.

The reservation that secondary structure in general exists in acid-denatured proteins invests the guanidine hydrochloride system with particular interest, for here, as Tanford and his associates have shown, ribonuclease (and most other proteins) behave as random coils, possessed of no detectable intermolecular interactions. Nevertheless, here too the unfolding gives evidence of intermediate states when followed by NMR at high resolution. Benz and Roberts (*FEBS Lett.*, **29**, 263; 1973) have found that the downfield resonance from his-12 begins to decay at lower denaturant concentration than those of the other three histidines. When denaturation is complete only one C-2 histidine resonance, corresponding to all four groups in the coiled chain, is to be seen. His-12 occurs in the N-terminal "S-peptide" region, which is very possibly the most susceptible to disruption. This result is in a sense at odds with the kinetic and thermodynamic features of this system, which meet the criteria for a grossly two-state denaturation process, but as Benz and Roberts point out, the deviation from such behaviour in structural terms may be very slight.

PSYCHOLOGY

Depression and the Pill

from a Correspondent

As many as one in fifteen British women on the contraceptive pill may find that it induces a state of depression which can be severe enough for the pill to be an unsuitable form of contraception for them. The reasons for this have been examined by a group at St Mary's Hospital Medical School, London, who have related the problem to a deficiency of vitamin B_6 (pyridoxine) in some women on the pill (P. W. Adams *et al., Lancet,* i, 897; 1973). It seems that treatment with vitamin B_6 may sometimes be effective.

The biochemical basis of this work is that the administration of oestrogens interferes with the metabolism of the essential amino acid tryptophan, increasing the amount metabolized by the nicotinic acid pathway. The metabolism of tryptophan involves the coenzyme pyridoxal phosphate derived from vitamin B_6, and therefore a pill-induced increase in the rate of metabolism of tryptophan uses up pyridoxal phosphate.

But one of the essential amines in brain function, 5-hydroxytryptamine, is also produced by a pathway which requires pyridoxal phosphate, and in patients with depressive illnesses an examination of the fluid surrounding the brain and spinal cord shows this amine to be present in abnormally low quantities.

Attempts have been made in the past to treat premenstrual depression in women on the pill with pyridoxine, but the importance of the work at St Mary's is that for the first time a double-blind crossover trial with pyridoxine has been made.

One of the problems in selecting patients for the trial was to ensure that the depression was a side-effect of the pill and did not have another cause. Most forms of depression give rise to symptoms of pessimism, tearfulness, anxiety and loss of sexual appetite, but also induce a disturbance of sleep pattern and appetite. These latter two symptoms are uncharacteristic of pill-induced depression, however, so women who had sleep and appetite disturbances or a history of mental disorder or obvious reasons such as marital disharmony that might lead to depression were excluded. The St Mary's team were then left with thirty-two women whose depression they felt to be caused by the contraceptive pill. Ten of these did not complete the trial, so the sample is small, but the results still seem significant.

The patients, divided into two groups, were treated with either 20 mg pyridoxine hydrochloride daily or a placebo and after two months the groups were interchanged. The women were on a variety of contraceptives though each contained 50 μg of oestrogen, and the length of time they had been on the pill made no difference to the results.

There was an alleviation of the depression in eleven women who at the start of the experiment were found to have an absolute lack of pyridoxine, but in the other eleven women whose biochemical assays were no different from other women on the pill the pyridoxine treatment showed no benefit over the placebo. So, as the St Mary's team point out, other causes must be sought for the depression in those women who did not respond to the pyridoxine.

Experiments along these lines may also conceivably throw some light on events in early pregnancy. Morning sickness has also been treated for some

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Magnetic Effect of Cofactors

The discovery that organic phosphates function as cofactors for haemoglobins, and are tightly bound to the deoxygenated state, has meant a re-evaluation of many physical properties, which were previously determined without regard to the presence and concentration of these ligands. The magnetic susceptibility of deoxyhaemoglobin, which is the subject of a re-examination by Alpert, Banerjee and Denis in next Wednesday's *Nature New Biology* (May 16), corresponds to ferrous haem iron atoms with an electronic spin of 2. This falls to zero in the liganded haemoglobin.

The classical value for the magnetic moment of deoxyhaemoglobin, obtained twenty-five years ago by Pauling and his colleagues, was about 5.4 Bohr magnetons. This turns out now to be the value for the complex with 2,3-diphosphoglycerate or inositol hexaphosphate. When the ligand is stripped off on a column, a drop to 4.9 Bohr magnetons is observed. This is exactly the theoretical value for independent spins. The higher magnetic susceptibility in the complex with cofactor must then be attributable to an added orbital contribution. The cofactor must therefore perturb the symmetry of the ligand field around the iron.

It is known from the X-ray work of Arnone that diphosphoglycerate binds in the central cavity of the protein at the junction of the two β chains, and in doing so induces displacements of up to 2 Å locally. Alpert *et al.* suggest that these can be sufficient to reduce the distortion of the ligand field around the iron from octahedral symmetry, which would have the effect of increasing the magnetic susceptibility. Both α and β chains probably contribute to this effect.

years with pyridoxine, so it may be that there is a relationship between morning sickness, increased oestrogen levels, and the depressions which can occur in early pregnancy.

HERPESVIRUSES

Genetics of HSV 1 and 2

from our Cell Biology Correspondent

THE genetic analysis of animal viruses, be they RNA viruses or DNA viruses, is still very much in its infancy but many animal virologists aspire after the achievements of bacteriophage geneticists and are seriously attempting to isolate suites of mutants that can be used in complementation and recombination tests. Animal virologists, of course, labour under severe disadvantages not shared by bacteriophage geneticists: animal cells grow slowly compared with bacteria, they are far more fastidious and to date nobody has detected suppressible mutants of animal viruses comparable to the amber and ochre mutants of phage. Temperature sensitive mutants of animal viruses, however, can be and have been isolated, and as Brown, Ritchie and Subak-Sharpe (*J. Gen. Virol.*, **18**, 329 ; 1973) take pains to stress, such mutants offer several distinct advantages which go some way towards compensating for their great disadvantage—their leakiness.

Subak-Sharpe and his colleagues have independently isolated nine 5-bromodeoxyuridine-induced temperature sensitive mutants of herpes simplex type 1 virus, which because of its large genome (about 105×10^6 daltons of double stranded DNA), presents the animal virologist intent upon genetic analysis with a formidable task. These nine *ts* mutants apparently fall into eight complementation groups and, therefore, presumably define eight herpes simplex 1 cistrons. Moreover, because eight of the nine *ts* mutants selected at random fall into different complementation groups, Brown *et al.* estimate that the herpes simplex 1 genome probably contains more than thirty cistrons which specify functions that are indispensable for viral replication at 38° C.

Using these nine *ts* mutants and a plaque morphology marker in three factor recombination tests, Subak-Sharpe's group has also produced a tentative linkage map of the herpes simplex 1 genome. The map is linear, locates nine cistrons—the eight defined by complementation tests and the plaque morphology marker—and spans about twenty-five recombination units. In short, the group has made a useful start to the genetic analysis of this large and complex virus.

With another colleague, Timbury, Subak-Sharpe has also begun to probe the range of genetic interactions between herpes virus type 1 and its relative type 2 (*J. Gen. Virol.*, **18**, 347 ; 1973). Making the most of their *ts* mutants of type 1 and type 2 viruses they have established not only that herpes simplex viruses types 1 and 2 can complement each other to a considerable extent, but also that the type 1 and type 2 genomes can recombine to produce stable recombinants. Furthermore, the progeny of cells infected with these two viruses include heterozygotes and in some cases viruses with a phenotype distinct from those of both parents. With the possibility that herpes simplex virus type 2 might be involved in the aetiology of human cervical cancer the Glasgow group should find it easy enough to justify extending this analysis.

Herpetologists will no doubt also have noticed Smith and de Harven's recent report (*J. Virol.*, **11**, 325 ; 1973) of what seems to be a neat method for concentrating stocks of herpesviruses, many of which do not yield high titres when they replicate in cultured cells. Apparently negative pressure ultrafiltration through dialysis tubing does the trick, by converting low titre stocks into high titre concentrates of intact unaggregated virus particles without loss of infectivity.

CELL FREE SYSTEMS

Translation *in vitro*

from our Cell Biology Correspondent

THE person who first manages to isolate strains of cultivated animal cell which harbour suppressor mutations and as a result contain suppressor transfer RNAs capable of translating amber or ochre codons in messenger RNAs will have achieved a triumph. The opportunity to isolate and exploit suppressible nonsense mutants of animal viruses is something animal virologists long after, and until suppressor mutant cells are isolated virologists must content themselves by working with temperture sensitive mutants that are a deal less satisfactory. The isolation of suppressor strains of animal cells is, it scarce needs saying, a formidable task that promises much hard and tedious work with no guarantee of ultimate success and, not surprisingly, nobody has embarked on it seriously. But thanks to the efforts of Schreier, Staehelin, Gesteland and Spahr (*J. Mol. Biol.*, **75**, 575; 1973) there is now at least an *in vitro* assay system that can be used to screen for the suppression of nonsense mutants in eukaryotic cell extracts.

Schreier and Staehelin (*J. Mol. Biol.*, **73**, 329; 1973) have recently devised a highly efficient mammalian cell free system in which, for example, during 40 min at 30° C, each ribosome will translate a globin messenger RNA molecule three or four times. While Gesteland and Spahr during the past several years have made themselves masters of *Escherichia coli* cell free systems which translate efficiently not only the genomic RNAs of RNA bacteriophages such as R17 and $Q\beta$, but also the messenger RNAs of T-even phages. These workers in collaboration have established that the mammalian cell-free systems of Schreier and Staehelin can, with appropriate modifications, be induced to translate faithfully the coat protein cistron and the RNA replicase cistron

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Messenger Translation in *Xenopus* Oocytes

A CAUTIONARY tale is told in a short contribution to next Wednesday's *Nature New Biology* (May 16) presented by Lane, Gregory, Iyatzumi and Scherrer. In recent years, evidence for the existence of messenger RNA sequences in high molecular weight RNA (HMW RNA) has been reported, based on experiments in which synthesis of globin or immunoglobin occurred following injection into *Xenopus* oocytes of HMW RNA extracted from the appropriate tissues (erythroblasts or myeloma cells). This made a neat and happy story, but now Lane *et al.* plead caution. The trouble seems to lie in the extreme sensitivity of the oocyte system as a test for mRNA translation. Thus, if as little as 1 per cent of the HMW RNA injected into oocytes is represented by contaminating aggregated cytoplasmic messenger, then synthesis of the appropriate protein would be detected. Even excluding cytoplasmic contamination, it is possible that nuclei, from which the HMW RNA is extracted, may contain functional mRNA.

The control experiment carried out by Williamson *et al.* (*Nature New Biology*, **241**, 66 ; 1973) to eliminate the possibility of 9S globin mRNA contamination of their HMW mouse erythroblast RNA is inconclusive. Williamson *et al.* demonstrated that no globin was synthesized when brain HMW RNA (extracted from a mixture of brain tissue and reticulocyte lysate) was injected into *Xenopus* oocytes. Lane *et al.* point out, however, that 9S mRNA in the lysate may have been degraded by brain enzymes. They suggest a control experiment to solve the problem. If HMW RNA were extracted from a mixture of duck erythroblast nuclei and rabbit reticulocyte polysomes then, if messenger aggregation does not occur, the HMW RNA should code for duck but not rabbit globin, whereas the 9S fraction from the same preparation should cause the synthesis of both globins.

of Qβ or R17 phage RNA. What is more, they have found that when the mammalian cell free system is programmed with RNA from the R17 amber mutant *amB2*, which carries an amber mutation at position 6 in the coat protein cistron, no coat protein is made. In other words, this amber mutation terminates faithful translation of the coat protein cistron in the mammalian cell free system just as it does in *E. coli* cell free systems. Obviously *amB2* R17 RNA can now be used to test cell free systems prepared from various animal cells by Schreier and Staehelin's methods to see if any contain an amber suppressor tRNA.

Virologists and others would also clearly like to have a cell free system which faithfully translates single stranded DNA from virus particles and cells because it is far easier to isolate and purify viral DNA than viral RNA. Since the mid-nineteen-sixties various groups have tackled this problem only to meet with disappointment. Bretscher (1969), for example, found than an *E. coli* cell free system programmed with phage fd DNA made a set of fMet dipeptides many of which were not related to known N termini of the phage proteins. Ihler and Nakada (1970, 1971), on the other hand, obtained more encouraging results: in their hands ribosomes appeared to bind to authentic initiation sites in T7 and T4 single stranded DNA. But according to Cardit, Goldberg and Steitz (*J. Mol. Biol.*, **75**, 449; 1973) ribosomes in conditions which allow optimal RNA-directed chain initiation in an *E. coli* cell free system, bind to single stranded DNAs from eight unrelated viruses or cells to yield predominantly three dipeptides fMet-Thr, fMet-Val and fMet $\frac{\text{Leu}}{\text{Ileu}}$. As they say, "although dipeptide synthesis primed by natural DNA templates may yet prove useful in deciphering the molecular basis of protein synthesis, it is probably of little value as a tool for decoding genetic information *in vitro*".

INERT GASES

Elastic Constants

from a Correspondent

THE adiabatic elastic constants of single crystals of neon at 24.3 K and of argon at 82.0 K have been determined by Stoicheff and his colleagues at the University of Toronto, using the Brillouin light scattering technique (*Phys. Rev. Lett.*, **29**, 1454; 1972). The results represent what is probably the most accurate set of elastic constant data currently available for the solidified inert gases, and they are important because they provide a sensitive test of recent theories of lattice dynamics. The measurements are particularly useful because they are made near the triple points, a region about which there are unresolved questions concerning the influence of anharmonicity and many-body forces.

The inert gases crystallize in face-centred cubic lattices in which the atoms are bound by the cohesive van der Waals interatomic forces. These forces are central and may be represented to a good approximation by simple radial potentials. Thus the solidified inert gases correspond to elementary models for the solid state and are therefore ideal for developing the lattice dynamics of simple solids. These theories have to be tested against experimental data for the bulk properties of the crystals. Most experiments so far on the solidified inert gases have, however, been carried out on polycrystalline samples and only recently has it been possible to prepare large single crystals. Some data for single crystals are now available for the lattice parameter, isothermal compressibility, thermal expansion and other thermodynamic properties; but to investigate properly the dynamical properties of the lattices, and hence the interatomic forces, accurate measurements of the elastic constants are also required.

Because of its small atomic mass and the consequently large amplitude of its lattice vibrations, neon provides a very stringent test of theory. Before the work of Stoicheff *et al.*, however, only one other determination of its elastic constant had been reported, based on neutron scattering on two crystals at 4.7 K. Argon, on the other hand, is the most thoroughly investigated of the solidified inert gases, both theoretically and experimentally. Even in the case of argon, however. there is considerable disagreement between the various experimental values for the elastic constants and a similar disagreement between experimental and theoretical data.

In spite of the improved techniques now available for preparing single crystals, accurate measurement of the elastic constants is difficult. Determinations of acoustic velocities in specific crystal directions have been made using neutron scattering and ultrasonic techniques. But so far these methods have yielded results which not only are mutually inconsistent but also imply a much lower elastic anisotropy for the crystals than can be accounted for theoretically. The principal difficulty with both neutron scattering and ultrasonic techniques seems to be that large single crystals (~1 cm^3) are required; these are hard to produce and maintain under the appropriate experimental conditions.

Recent advances in high resolution Brillouin spectroscopy now allow values of adiabatic elastic constants for crystals to be determined with high precision by this technique. The application of the method to the solidified inert gases has two important advantages over the alternative techniques: first, because it only requires small samples (~1 mm^3); and, second, because light scattering studies do not require a transducer or other device to be physically bonded to a crystal sample.

Stoicheff *et al.* have grown crystalline samples of argon and neon in the tail sections of suitable dewars and have checked orientation and quality of the crystals by means of Laue X-ray transmission photographs. Brillouin spectra were recorded for the crystals at several different orientations, using a single frequency argon ion laser and a piezoelectric scanning interferometer. The observed Brillouin spectra were found to contain the longitudinal component and one "slow" transverse component. The reason for the non-appearance of the expected "fast" component is that it is generally of extremely low intensity in the solidified inert gases, except for a limited range of orientations not included in the present arrangement. Each of the frequency shifts, ν_i, was subsequently analysed using the usual Brillouin equation

$$\Delta\nu_i = \pm 2\, \nu_i\, (V_i/c)\, n \sin(\theta/2)$$

to determine the velocities V_i of the thermal waves.

Three independent adiabatic elastic constants C_{11}, C_{12} and C_{44} describe the elastic properties of a cubic crystal. Values of these constants accurate to about ±4% were deduced from the velocities, together with values for the adiabatic bulk modulus and the Grüneisen parameter.

The results for neon at 24.3 K are all 25–40% lower than the only other set of data available for neon, at 4.7 K. This suggests that anharmonicity is very important in neon. The results for argon differ quite considerably from earlier investigations reported, particularly from measurements based on ultrasonic techniques. The reason for this is presumed to be because earlier measurements have been carried out chiefly on polycrystalline specimens. The great value of the present method lies in the knowledge that the samples were single crystals of well defined quality and orientation.

It is somewhat surprising to find that the experimental values of C_{11} and C_{12} still differ from the calculated values by about 1.5 times the estimated uncertainties. This is, however, much better agreement than has generally been found in the past. At this stage, it would be very useful to have further measurements on argon and neon, particularly at different temperatures.

GRAVITATIONAL RADIATION

Waving Goodbye to Weber's Waves

by our Cosmology Correspondent

WEBER'S gravitational wave detectors may well be detecting something—but it is almost certain that they are not detecting pulses of gravitational radiation. This was the clear consensus that emerged from the Oxford–Cambridge–London Relativity Seminar, held at All Souls College, Oxford, on April 27. Theoretical discussions formed an important part of the seminar, particularly those concerned with the possibility of detecting black holes and the implications of the discovery of Cyg X-1, a binary system which contains the best candidate yet for such a completely collapsed object. But the proceedings were dominated by the experimentalists and their reports of the present status of the search for gravitational radiation—a remarkable turnabout from the situation a couple of years ago, when it would have been laughable to suggest that such a relativity seminar could be so concerned with experiments.

Dr R. W. P. Drever (University of Glasgow) summarized the present situation in the experimental field. All the present crop of experiments were, of course, inspired by Weber's claims that pulses of gravitational radiation were being detected by his equipment. Others have found it very difficult to duplicate Weber's results, and questions regarding the sensitivity of his equipment, and the possibility of unconscious human bias, have inevitably arisen. A team from the Soviet Union were the first to follow Weber, using a detection system which had the advantage that it could be accurately calibrated.

Basically, all the gravitational wave detectors consist of massive aluminium cylinders, cooled to reduce thermal effects and suspended in a vibration-free environment. Weber's equipment has never been calibrated directly; he estimates its sensitivity as better than 1 kT, but others have estimated the response of this apparatus as only sensitive to 10 kT pulses. The Soviet instrument is calibrated as sensitive at the 9 kT level, so some doubt remains as to whether that instrument's negative result should be directly compared with Weber's positive results.

What of more sensitive detectors? Basically, there are two approaches now being employed: bigger detectors and/or more sophisticated signal processing. Neither approach has met with success in the form of a definite detection of even one pulse of gravitational radiation. At the University of Bristol, what is essentially a second generation experiment, in terms of sensitivity, is still being constructed. There, the attitude is that enough people around the world are already producing negative results at the few kT level, and that it is worth waiting a little longer to produce a machine more sensitive by five orders of magnitude. In Glasgow, a detector rather smaller than Weber's but with about the same sensitivity is one of the many producing negative results, and, perhaps most interesting of all at present, a joint Munich/Frascati system, designed to be as similar to Weber's as possible, is also failing to produce any positive results.

The conclusion, from these and other experiments, seems inescapable. Weber has not found pulses of gravitational waves. Dr Drever speculated that perhaps Weber's pulses are associated with disturbances in the Earth's magnetic field, because his apparatus is unique in containing large amounts of ferrous material, although the detector cylinder itself is non-magnetic. It is also still just possible that Weber has been fortunate in building a detector which operates at a favoured frequency for gravitational radiation, but this faint prospect did not stir any enthusiasm in Oxford.

The theoreticians were far from displeased to learn of these negative results, because according to general relativity sensible disturbances, such as supernovae explosions, should produce gravitational radiation detectable on Earth only with equipment much more sensitive than Weber's. Professor M. Rees (University of Sussex) estimated that an improvement by a factor of 10^8 is needed before positive results can be expected, because this would extend the range of the detector to the Virgo cluster and offer hope of detecting one supernova a year. Professor D. Lynden-Bell (University of Cambridge) turned the question around to ask at what level of sensitivity relativists would be distressed to learn of the continued failure of the experiments to find gravitational waves. It seems that the pundits will only begin to worry when negative results are obtained by experiments 10^{12} times more sensitive than those of today.

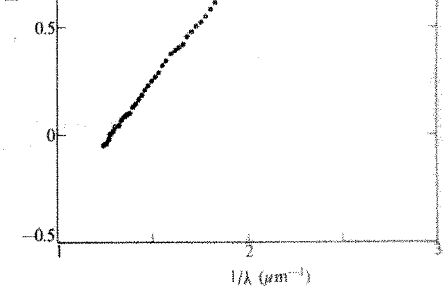
That might have seemed to have brought the young science of gravitational wave experimentation to an untimely demise; but Dr Drever promptly returned to the fray to suggest that at the present rate of improvement in techniques the first quantum of improvement, by Professor Rees's factor of 10^8, could be achieved within a few years. As well as the ultimate refinement of the Weber approach, a 5 to 6 ton cylinder levitated by superconducting magnetic coils and maintained at 1/30 K, with tiny oscillations detected by Josephson junction effects (with instru-

Reddening of Stars near the Galactic Centre

IN next Monday's *Nature Physical Science* (May 14) Whittet, van Breda and Nandy describe interstellar extinction curves which they have derived from photoelectric scanner observations of twenty-two early type stars in a region of the southern Milky Way which includes the galactic centre. There are considerable variations in the data, which are not correlated with position of the stars in the plane of the Galaxy, suggesting that the extinction law in these regions is strongly influenced by the physical conditions which prevail in local dust clouds.

The scanner observations were made with the 36-inch telescope at Cerro Tololo during May 1972. They covered 3480 to 5240 Å with a bandwidth of 40 Å in the second order, and 5160 to 8040 Å with a bandwidth of 80 Å in the first order. The area surveyed was from $l^{\mathrm{II}}=260°$ to $l^{\mathrm{II}}=30°$, passing through the galactic centre.

The twenty-two reddened stars examined seem to fall into six chief groups, as far as the extinction curves are concerned (see figure). But the stars in each group are not spatially related—for example, stars which contribute to extinction curve D include one at $l^{\mathrm{II}}=343°$, one at 15°, one at 263° and three others. Even this distinction is somewhat artificial, however, and Whittet *et al.* point out that there seems to be a continuous family of curves, at least between curves A and D in this figure. Of the nine stars observed in the galactic centre region "six show a reddening law close to curve C; the other three follow curves A, B, and D".



Characteristic extinction curves for reddened stars observed at Cerro Tololo.

ments being developed jointly by a Stanford/Louisiana State University/Rome group), there is also the prospect of experiments in space with masses a long distance apart, distances being monitored by laser interferometry. Weber may not have detected gravity pulses, but by inspiring the work of the past two years around the world he has ensured that there is now a real prospect of making the final push to produce detectors sensitive at the levels which relativity theory suggests will be suitable for the detection of such radiation. Just as the steady state theory provided the incentive for work which ultimately led to convincing evidence in favour of big-bang models, so Weber's incorrect interpretation of the events detected by his apparatus may prove the first step in the detection of pulses in line with relativity theory.

TROPICAL ECOLOGY

Fire and Savanna

from a Correspondent

IN 1952 P. W. Richards (*The Tropical Rain Forest*, Cambridge University Press) expressed the general view that the savanna grasslands of Africa, and their recent rapid spread south of the Sahara at the expense of closed forest, are wholly ascribable to the increased incidence of man-induced fire rather than to climatic conditions and a recent increase in aridity. These African savannas have been described as the largest area of mismanaged land in the world, yet fire can be exploited judiciously as a management tool. On April 9 the Tropical Group of the British Ecological Society met to discuss recent research on the effects of burning on savanna vegetation and animal populations.

Dr R. Rose-Innes (Land Resources Division, Overseas Development Administration) described how firing early in the dry season induces a mild burn favoured by silviculturists; the intense late burns encourage grassland at the expense of forest, and burns after the first rain can destroy the woody vegetation as it begins to shoot. Dr. R. M. Lawton (Land Resources Division, ODA) elaborated the use of fire in forest management; Dr R. H. Kemp (Commonwealth Forestry Institute, Oxford) pointed out, however, that savanna regions could provide 10–20 times their present yield per hectare of hardwood timber if planted with tropical pines. The use of photogrammetry for interpreting patterns of savanna burning in relation to climate, soils and agricultural practice was summarized by Dr R. P. Moss (University of Birmingham), and Dr A. Blair Rains (Land Resources Division, ODA) described its use for estimating grazing resources in the semi-arid regions of Southern Africa where grass is too sparse to support a fire detrimental to woody growth.

Professor M. R. Delany (University of Southampton) and Dr B. R. Neal (University of Saskatchewan) studied the effect of fire on small rodent populations in Uganda's Queen Elizabeth Park. Twelve species were found, if considered by three size categories, to occupy specific niches defined by microhabitat, food resources and feeding behaviour; responses to fire differ, but there is an initial increase in numbers, after which a drastic decline and eventual recovery follows. Small mammals favour the cover of tall grasslands, and their numbers and diversity are inversely proportional to those of large mammals which favour the more nutritious short grasslands.

Contributions by Professor D. H. N. Spence (University of St Andrews) and Dr G. Harrington (CSIRO, Canberra) on burning and grazing in relation to deterioration of habitat diversity in two Ugandan national parks emphasized the impression gained at the meeting as a whole: fire, the old scourge of African savanna, is being overtaken by the new spectre of overgrazing, especially by elephants. In parts of East Africa fire exclusion leads to no appreciable amelioration of deforestation unless grazing is also excluded; the old traditional use of fire-arms, and the prevalence of tsetse, in West Africa has so far prevented overgrazing, but with the elimination of tsetse it can be anticipated. Dr Kemp's dream of productive softwood forestry in African savanna therefore remains a long way off, while in the Brazilian savannas the new highway is leading to a rate of forest devastation never before witnessed.

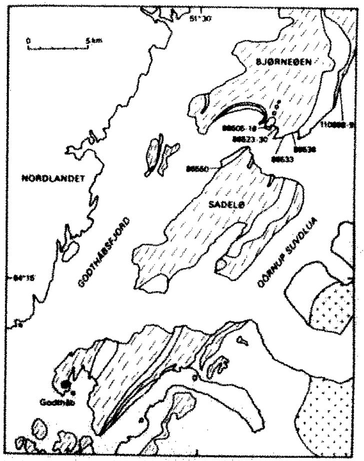
More Very Old Rocks from Greenland

THE oldest known rocks in the world are quartzofeldspathic gneisses (the Amîtsoq gneisses) from the Godthaab area of West Greenland which were dated in the range 3,700–3,750 million years by members of the group at the University of Oxford in collaboration with the Geophysical Survey of Greenland (Black *et al.*, *Earth Planet Sci. Lett.*, **12**, 245; 1971 and Moorbath *et al.*, *Nature Physical Science*, **240**, 78; 1972). In next Monday's *Nature Physical Science* (May 14), members of the same team (Pankhurst *et al.*) report further rubidium–strontium ages from this part of Greenland, although the new ages represent a more recent event in the evolution of the Godthaab area.

The rocks in question are the Nûk gneisses which lie in close association with Amîtsoq gneisses but which occur as dykes and subconcordant sills cutting the older rocks and as large subconcordant complexes. Most of the samples now dated by Pankhurst and his colleagues are from a large polyphase complex of quartzofeldspathic rocks in which the earlier phases are dioritic and the later ones granitic. The interpretation given for the Nûk gneisses is that they are derived from a syntectonic suite of calc-alkaline igneous rocks which were intruded into the Amîtsoq gneisses and the Malene supracrustals (metavolcanics and metasediments) which postdate them. Conversion to gneisses then took place by metamorphism and deformation both during and subsequent to, the intrusion of the parent rocks.

The Nûk gneisses dated are from the north of Godthaab where they are metamorphosed to the same degree as the associated Amîtsoq gneisses and Malene supracrustals. The metamorphic grade is, however, significantly lower than that to the south of Godthaab where the 3,700–3,750 million year old Amîtsoq gneisses were obtained. For this reason the rubidium–strontium isochron age for the Nûk gneisses is thought to represent the time of crystallization of the igneous parent material rather than the time of later isotopic rehomogenization associated with metamorphism.

The age obtained by Pankhurst *et al.* is 3,040 ± 50 million years, which gives another fixed point on the time scale for the crustal evolution of West Greenland. Thus the plutonic development giving rise to the parent rocks of the Amîtsoq gneisses, the conversion of the parent rocks to gneisses, the intrusion of a basic dyke swarm (the Ameralik dykes) into the gneisses, the production of the Malene supracrustals, and the intercalation of the Amîtsoq gneisses and the Malene supracrustals, must all have taken place in the interval from about 3,700 to 3,000 million years ago.



The Godthaab region of West Greenland. The Nûk gneisses are represented by diagonal dashes, the Amîtsoq ones by the stippled pattern.

"Backcasting" with the World Dynamics Models

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Two members of the Science Policy Research Unit at the University of Sussex discuss whether or not a Systems Dynamics model can meaningfully be run backwards in time.

THERE seems to be some misconception about both the feasibility and the utility of running a System Dynamics model backwards in time (see, for example, Dennis Meadows's response to the Science Policy Research Unit's critique of his World Dynamics model in *Futures*[1]). That issue forms the principal substantive technical criticism of our work made by Meadows. By clarifying this issue once and for all, we would hope that arguments about methodological technicalities, although important, will not be allowed to obfuscate the very important and more fundamental criticisms of his work which are made in the *Futures* articles.

Equating

With regard to the issue of feasibility, it is instructive to consider the kind of equations generally used in such models, and the circumstances in which it may not be appropriate to "backcast" using such a model. System Dynamics models comprise a set of non-probabilistic, non-linear first order difference equations. Using the Dynamo programming language they are solved numerically over discrete time intervals. What one is attempting to do by this is to approximate to the continuity of the set of assumed mechanisms which make up the model. The equations may include feedback processes (that is, the rate of change of a variable depends at least in part on its own value) and delays (that is, a change in a variable does not instantaneously affect any others). The equations used to introduce delays in the System Dynamics method also take the form of first order difference equations (they do not represent true delays). As the original system is assumed to be continuous there is no mathematical reason why the properly posed model should not run as well in the backwards direction as it does in the forwards direction. Provided that the parameter values are correct, any inability to reproduce transients when calculating through the same time period from opposite directions must therefore arise as a result of the approximations made during the computer calculation of a System Dynamics model using the Dynamo language.

In the first place it should be pointed out that the order in which computer calculations are carried out is not perfectly reversed when the sign of the time step used in the calculation is switched. In the forward calculation using, for example, a step length between successive calculations of one year, parameter values for the year (say) 1930 are determined from those at 1929, whereas in the backwards direction they come from those at 1931; because in most dynamic systems rates of change are not constant, this leads to asymmetry when calculating in opposite directions. Provided the step between successive calculations can be made small enough, any errors of approximation (as opposed to numerical errors) arising from this ought always to be insignificantly small. If, however, one reduces errors at each iteration by reducing the time increment to a very small value, the total number of iterations is increased and sooner or later numerical errors will still accumulate adversely. A related problem arises when numbers generated by the equations of the computer model are of the same order of magnitude as the rounding errors involved in the numerical computation (the Dynamo language reads numbers to five significant figures only, although it calculates to more). This can occur, for example, when the difference between two very nearly equal numbers is taken. In such cases the numerical solution to the equations of the model will not agree with their analytical solution. The level of agreement needed depends on the problem, but certainly the numerical accuracy should be such as not to mislead. It is extremely important, therefore, in the development of a dynamic model to ensure that at no point in the calculation are solutions excessively interfered with by either numerical or approximation errors. Further, unless this is assured, solutions calculated through a particular time period from opposite directions will not be the same. If these aspects are properly attended to there should be no reason why trends evaluated in the forwards direction should be any closer to the analytically derived solution than trends calculated in the reverse direction.

There is, however, a further danger if the solutions of a dynamic model are matched to data from a period in which only monotonic trends have been observed; potential errors are automatically built into the model, for parameters must be "adjusted" to account for the systematic biases of approximations in the calculation. Consequently when the model is reversed or used to forecast into a period for which the "adjustment" is no longer valid, approximation errors may be more prominent. As an aside it should be pointed out that in most System Dynamics models where transients are not monotonic, individual relationships are actually taken as time reversible even when calculating in the forward direction.

There are, of course, circumstances in which a dynamic model should not be expected to run backwards. For instance, it is well known that certain difference equations have different numerical stabilities depending on the direction of calculation (for example, Bessel's difference equation for some parameter values). Another example would be a model in which any process within the model has decayed as a result of forward running to the extent that errors of approximation are already affecting parameter values. In some models, but not in the System Dynamics models known to us, transients are continually decaying and being replaced by others (for example, models of turbulent flow which are usually representations of a steady state) and here it is not appropriate to reverse the time direction of the numerical calculation. For this reason, it is certainly not correct, as Anthony Tucker has done[2], to draw analogies between System Dynamics models and models of aircraft flight.

With regard to the utility of running a System Dynamics model backwards, the following may be said. As a model is a mimic of reality, it should reflect mechanisms and trends for the period of interest. If the same mechanisms were important in the past (before that period of interest), it is only reasonable to expect that a model should "backcast" or extrapolate into the past. In some sciences, for example, astronomy, retrodiction is as important as prediction. To argue that one may not backcast using a model is to say that mechanisms were in fact different in the past. This is an essential part of the scientific paradigm and applies equally in both the physical and the social sciences (see ref. 3). To argue that one should not attempt to backcast using a System Dynamics model, while at the same time admitting that the same mechanisms operated in the past, is to say that such models are beyond the reach of normal scientific criticism. The same can be said of a model for which the individual relationships are taken as reversible in the forwards direction. If one accepts that System Dynamics models are amenable to normal scientific criticism then "backcasting" clearly has a certain utility. In a non-linear dynamic model containing many parameters and many feedback loops, it is not practical to make all possible tests on the reasonableness of the model. "Backcasting" can be expected to drive some variable to unrealistic values and hence show up inadvertently incorrect assumptions underlying a model. Besides this, successively running a dynamic model forwards and backwards about a given point in time will point to any inconsistent assumptions and numerical instabilities within the model. This technique has been successfully employed in the construction of the ILO Bachue model[4].

World Dynamics Models

In the light of the above discussion, it is now pertinent to turn to the World Dynamics models of Jay Forrester and Dennis Meadows and the ongoing debate between Meadows and the Science Policy Research Unit (SPRU). In our analysis at SPRU of Forrester's and Meadows's System Dynamics world models, we examined the performance of the models when run backwards in time from their starting date (1900). The reasons for doing this were as follows: the (World 2) model exhibits unrealistic population growth in the immediate post-1900 period (even though its parameters have been adjusted to match 1900 to 1970 world trends). This was not apparent from the publication *World Dynamics*[5] presumably because of the coarse scale used to illustrate Forrester's runs of the model. The population figures calculated at 1904 are actually lower than those calculated at 1900. At 1900 the assumed birth and death rates are reversed in magnitude. Their values are 48 per thousand and 55 per thousand respectively which compares rather poorly with the accepted 1900 figures of about 32 and 20 for the developed regions of the world and 40 and 35 for the under-developed regions[6]. It is clearly instructive to extrapolate these apparent errors to an earlier period to see where the trends generated by the model lead from. Further, as already pointed out, running a model backwards can give some indication of its reliability in the forward direction.

As explained in *Futures* and in ref. 7 running World 2 backwards to before 1880 proved impossible, because of instabilities arising principally from poorly estimated parameters used in the calculation of birth and death rates. For obvious reasons only the essence of our argument concerning this effect was presented in *Futures*, although it was fully communicated to Meadows at a date well before publication. In view of Meadows's preoccupation with this technical detail, however, we are obliged to discuss the matter further. But first, there are a number of "red herrings" to be dealt with. Meadows says, for example, that "reversing the sign of the solution time increment to make a feedback model 'run backwards' must radically alter the entire dynamic character of the model. With a negative time increment, negative loops are converted into positive ones (for example population will be augmented by deaths and depleted by births)". We fail to see the relevance of this obvious truism. He says "delayed relationships will also be asymmetric in time under some circumstances" and that this is another factor which makes backcasting with the models "completely meaningless". The fact is, however, that there are no explicit delays in Forrester's World 2 model. Those in World 3 may be reversed in the same manner as other equations in the model simply by making the sign of the time increment negative. Furthermore, Meadows says, "stochastic influences on the behaviour of a model in the forward direction will not, in general, be recaptured in the reverse mode". But the fact is that neither Meadows's nor Forrester's world models are probabilistic. None of the equations account for random events. Similarly Meadows's remarks about our forward projections with the World 2 model from 1850 do not apply to what we actually did.

Meadows says (in his response in *Futures*), "disequilibria introduced through the assignment of initial values may insert transients in the model's behaviour which cannot be reproduced in the reverse direction". The assignment of inconsistent initial values can cause confusion in dynamic models, especially those pertaining to the social sciences where in general theories and data sources are relatively weak and often inconsistent. Inconsistency between the initial values given to the parameters of a dynamic model and the set of equations which link them may arise. In successive iterations of the dynamic calculation the parameters will adjust to the form of those equations and the model will "settle down". Where the equations and parameters are inconsistent in the same sense that a set of simultaneous equations are inconsistent "settling down" can be rapidly achieved by beginning the iteration with a very fine time increment. The effects of wrong structural assumptions within a model, on the other hand, are not removed by this technique (see the discussion of the World 2 population minimum in *Futures*).

Sussex Experiment

In the Sussex experiment the World 2 model is run backwards from 1900 and forwards again through 1900 giving the same qualitative results at each pass. The calculation of "pollution" exhibits numerical instability when running in the backwards direction and Meadows shows that in attempting to run the World 2 model backwards from 1940, errors of the order of one part in 10^5 in the initial values (that is, of similar order of magnitude to the Dynamo approximation errors) can cause the model to be unstable. He implies that this same instability invalidates our comments on "backcasting" from 1900. This is simply not the case—although the instability is present it drives the net pollution to negative values which are then taken as zero, thus effectively decoupling pollution from the rest of the system. The insertion of more realistic values of pollution had only very slight quantitative effect on the behaviour of the other parameters. Meadows has shown that small biases can be introduced into the pollution sub-system when backcasting from 1940 which can result in pollution taking large positive values and that this drives other parameters to abnormal values. Unfortunately we are unable to reproduce the effect shown in Meadows's response without the introduction of such biases. He might equally well have shown runs with an opposite bias to the pollution system. In this case the model would retrace its population trajectory backwards through 1900 and the retrodicted population "explosion" in 1880.

Finally, on this point, Meadows says that if the state variables are entered with "absolutely no error" (as with the technique adopted originally with Bachue) then World 2 can backcast its own behaviour over limited periods even from 1940. (Running the model backwards from 1900 poses no problem in this respect—the initial values are identically those used for the forwards run.) Whereas the instability in the backward run from 1900 is shown to derive largely from wrong assumptions in the model, that at 1940 comes from numerical errors in the computation. The variables which are most affected are POLG (pollution generation) and POLA (pollution absorption). These are calculated quite independently. In 1940 these take the values 1.737 and 1.701 units respectively, so changing the numerical values of any one of the several parameters used to calculate them by about 2% can turn a net pollution increase into a decrease when calculating in the forward direction. As no information of such accuracy is available for "pollution" (see, for example, Marstrand and Sinclair in chapter 7 of ref.)7 the population collapse resulting from the accumulation of pollution which is "predicted" by the model must be viewed with some reservation.

In *The Limits to Growth* Meadows[8] says "... every assumption we make is written in precise form so that it is open to inspection and criticism by all" and "implications [of assumptions] can be traced without error by a computer no matter how complicated they become". To the first statement we would comment that the equations of Meadows's model (World 3) were still not generally available one year after his book was published. To the second we would argue that it is the responsibility of the modeller of a complex system not only to show awareness of the hazards of computer modelling but also to demonstrate that they do not invalidate the conclusions which are drawn.

By answering Meadows's criticism and by drawing attention to the issues involved in a particular aspect of modelling, "backcasting", we are pointing to some of the difficulties and dangers inherent in building complex computer models. Finally, we would reiterate our hope that the arguments can now turn back to the more fundamental issues.

[1] *Futures*, **5**, No. 1 (1973).
[2] *The Guardian*, March 7 (1973).
[3] Brown, Robert, *Explanation of the Social Sciences* (Routledge, London, 1963).
[4] *Nature*, **241**, 160 (1973).
[5] Forrester, J. W., *World Dynamics* (Wright Allen Press, Cambridge, Mass., 1971).
[6] *A Concise Summary of the World Population Situation in 1970* (United Nations, 1971).
[7] *Thinking about the Future* (Sussex University Press, 1973).
[8] Meadows, D. L., *et al.*, *The Limits to Growth*, 22 (Universe Books, New York, 1972).

Mathematical Literacy for the Non-mathematician

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This article is based on an address by Dr Steiner at the annual award ceremony of the Guinness Prizes in Mathematics and the Sciences in July 1972.

THERE is a well known passage in a letter of Keats in which the poet says how ardently he would wish to know precisely how Shakespeare sat or moved about the room when he was composing "To be or not to be" or another of his immortal soliloquies. I have often wondered how R. Clausius sat when, in a paper on thermodynamics of 1852, he gave the first definition of entropy. The concept had been implicit in the study of caloric theory since Sadi Carnot's *Reflections* of 1824. The mathematical bases had been worked out in Clapeyron's *Memoir on the Motive Power of Heat* ten years later. Kelvin had grasped and publicized the importance of Carnot's analysis. But it was Clausius who, with a characteristic German bent for the total metaphysical picture, drew the epochal conclusion. The Universe is running down; the increase of entropy is ineluctable. What lies ahead is, in Clausius's later term, a "heat death" or the inertness and randomization of total thermal equilibrium. The notion that the Universe will end either in conflagration or lifeless cold, is as old as mythology. Structurally it probably derives from the fact that our intellect is scarcely able to conceive of a beginning without an end. But until Clausius, the force of the idea was symbolic; it belonged to the domain of apocalyptic or messianic clichés. In Clausius's papers on thermodynamic exchanges and the theory of gases, the image of an end to our universe takes on the quiet certitude of an algebraic function. It is seen not as a religious mystery or philosophical metaphor: but as a mathematically verifiable truth (and the necessary algebra is, in fact, of an elementary elegance, deriving ultimately from $e = T \log v$, where e is the quantity of heat needed to maintain the temperature of a gas during dilatation, T the temperature in degrees and v the volume in litres). In short, Clausius wrote down the proposition, and the proof of the proposition, that there will be an analytically demonstrable, analytically foreseeable end to time and to the cosmos. Did he put down his pen for a moment or go to the window and look out?

It would be foolish to set the one moment above the other. Clausius's sentence generates the magic of exact truth. The precisions and truths in a Shakespeare soliloquy are of another order. Keats himself felt that the two classes could be compared and, in a somewhat gnomic passage, makes of "truth" an eagle higher than "poetry". But it is more useful to see at how many points the two modes of achievement correspond. Both represent the ultimate pressure of the human mind on reality, the capacity, indeed the compulsion, of consciousness to generalize beyond the discrete fact, to construct imaginatively and to impose on the world a living shapeliness. The poet and the mathematician are the great refusers of disorder. They use language—and mathematics is, above all, a language—to form constructs of meaning, of organic relation, of rational prediction without which our sensibilities would operate at those monotonous levels of instinctual immediacy, of constantly awed acceptance which seem to delimit the mental existence of animals. Poetry, philosophy, mathematics are the counter-statement of man to the brute determinism of the organic condition. We owe to them the best of our dreams. In poetry and mathematics, moreover, the key to major forms is one of disinterest. G. H. Hardy's axiom of "uselessness" is an extreme statement. The "uses" of poetry and of even the purest mathematics are manifold, but of a special kind. They provide the psyche with free spaces, with an indispensable sensation of freedom in the face of biological or social constraints. A play by Shakespeare, a paper by Gauss, are an essential reminder of the freedom of thought from the constraints of reality, from the demands of mere survival. This last point is perhaps the deep link. The great poet gambles on transcendence, on the uncanny power of words to live far longer than those who have first spoken them. Clausius affirms that the human intellect can, via the unsparing dignity of consequent reason, foretell an end to the universe which it inhabits. No less than the artist, the mathematician can answer with Jacobi, that he practices his craft "for the honour of the human spirit", that he comes as near as we can to showing the triviality of death. Hence a quality of delight common, vital to both. "If I was smitten by algebra", wrote Edgar Quinet, "I was dazzled by the application of algebra to geometry. . . . The idea, the possibility of expressing a line, a curve, in algebraic terms, by an equation, seemed to me as beautiful as the *Iliad*. When I saw this equation function and solve itself, so to speak, in my hands, and burst into an infinity of truths, all equally indisputable, equally eternal, equally resplendent, I believed I had in my possession the talisman which would open the door of every mystery." The "zero at the bone" should be the same when first we hear a great line of verse—St John of the Cross's *Oh noche amable más que el alborada*—and when we first grasp Cantor's proof that there is no greatest cardinal number. In either moment, the mind has stretched and altered the measure of man.

It is when the two modes interact that culture acquires a special lustre and coherence. Precisely this interaction characterizes the two seminal moments in western intellectual history, the flowering of ancient Greece and the period from Galileo and Descartes to Newton. It is impossible to separate the Greek discovery of irrational numbers from the unexcelled sense of Greek tragedy and poetry for the complexity and metaphoric structure of human relations and of man's place in the world. Conversely, it would be worth investigating what psychological, conceptual affinities there may be between certain failures in Greek political maturity and economic order and the inability of Greek mathematics to arrive at a genuine abstraction of space. The seventeenth-century duality of mathematics and philosophy, of mathematics and the arts, is crucial to modern civilizations. It was embodied in individuals such as Descartes, Kepler, Fermat, Pascal, Boyle and Leibniz. It permeated the confident rigour of philosophic and poetic language, the sense of a commanding at-homeness in natural and social experience which gives to the thought of a Galileo and of a Newton an unmistakable centrality. The music of the baroque, Vauban's military architecture, the mechanics of Huygens and the Bernouillis, exhibit a parallel stylishness, an economy of line and tension, which are functional in the general and in the algebraic sense. Today also, there are exceptionally gifted individuals in whom a mathematical and a humanistic sensibility are congruent. There is a subtle current of mathematical education and continued interest in some of the most interesting of modern literature: in novelists such as Musil and Broch, in the aesthetics of Valéry, in the poetry of William Empson, in the fantasies and "permutations" of Raymond Queneau. There is an explicit mathematical strain in certain schools of contemporary, totally programmed music and in kinetic art. Symbolic logic and linguistic analysis draw on modern algebra and on set theory. But the harmonic strengths of interaction, of concordant creation which mark the role of mathematics and of the arts in fifth-century Athens and seventeenth-century western Europe, have largely disappeared. Why should this be?

The common-sense answer is that mathematics have become so difficult that only the specialist can hope to grasp any but their rudiments. It is no longer possible to be both humanistically or generally and mathematically literate at the same time. There is a psychological puzzle here. When was mathematics "easy"? Was Apollonius on conic sections "less difficult" for his contemporaries to understand than were, say, Fermat on prime numbers or Dirichlet on the application of Fourier analysis to number theory? In other words: is "difficulty" not, in part at least, a cultural, almost aesthetic criterion with its own relativism and psychological history? "In part, at least" is probably the crucial factor. As mathematics progress, a very sharp increase in intrinsic difficulty does seem to occur. If I referred, a moment ago, to Fourier series, this was not entirely accidental. It is with the Fourier series, and with Poisson's combination of the Fourier series with the Fourier integrals, that analysis, which is in turn the foundation of modern mathematical reasoning, seems to undergo a quantum leap of difficulty. I would almost want to express the change in temporal coordinates: the time-span of attention needed to grasp an analytic proof for the so-called Gauss Reciprocity Law or the Kronecker integral which allows an analytical treatment of spaces of more than three dimensions, is longer, more severely abstractive from the facilities of ordinary random consciousness, than any called for by Euclid or even by Descartes's analytical geometry. Indeed, nothing in man's history is more fascinating than the capacity of the intellect to pose mathematical problems which are at first insoluble but whose postulates carry with them both the obligation and possibility of subsequent solution. The tree literally grows as the roots deepen into the unknown. But whatever the contingent and inherent reasons, the place of mathematics in our general culture has become one of specialized isolation. The consequences have, I believe, been more damaging than we usually realize. The sense of fragmentation in our understanding of natural and social phenomena, which is a cliché of current self-consciousness, may well have its source in the widening split between a mathematical and a humanistic sensibility. This may be so in a very simple, statistically-distributive sense: modern mathematics probably enlists intellectual and spiritual resources very close to the top of the human curve; by drawing them off into a remote, highly insulated sphere of performance and mutual stimulation, it leaves the rest of our creative, intellectual reserves significantly impoverished. There is no direct feedback. But the problem is also one of the more general climate of imagination, of the habits of feeling which underlie our political and social conduct. Mathematics is a sober, fiercely self-critical enterprise; but it has, since Plato, hobnobbed with eternity. It is a deeply individual, almost private obsession; but it is also inevitably collaborative and the intense competition is one that is harnessed to a common, transcendent pursuit. Mathematics demands a unique degree of consistency and verifiability; but uniquely also, these criteria will frequently coincide with the unmistakable, though very difficult to define, ideals of beauty, of perfected shape. However complex and particular in formal focus, mathematics is universal, its grammar belongs to no single race or *milieu*. Consider these contrastive but correlative forces of sobriety and philosophic height, of individuality and teamwork, of verification and beauty, of local style and universal access—and you will have a shorthand list of some of the energies and qualities of feeling most lacking in the present tenor of our private and political existence. It cannot be wholly a coincidence that a society so vulnerable to boredom, to every type of intellectual and political mendacity or simplification, so trapped in the insistent chaos of the moment, should also be a society which is, mathematically, largely illiterate. The intimation that a city without musicians and geometers (related guilds) is a poorer, more threatened place, is as old as Pythagoras.

What can be done?

It is all too obvious that my own remarks are those of a mathematical illiterate, that I am myself a product of a largely split educational tradition. Those present here tonight will have a much more acute, responsible view of the educational problems than any I can show. All I can offer therefore is anxious generality. But if my preceding remarks have any merit, it may be that mathematics, like certain other human pursuits, is too serious a matter to be left only to mathematicians or even to teachers of mathematics.

A great deal of effort and intelligence (the Guinness Awards are a case in point) has gone into the revision of the school syllabus in mathematics, into the study of better ways of teaching and organizing mathematical skills in class. But so far as England goes, the resulting improvements have not affected the real problem: which is the premature fission between the sciences and the arts, between mathematics and the humanities. O-level mathematics are notorious for their low standards, for the fact that one can get by on almost nothing but a bit of expedient drill. Yet it is at O-level, often even before, that a major proportion of young people in England bid a lasting goodbye to mathematics. In short, mathematical illiteracy is not only condoned by implicitly facilitated and, I would nearly say, encouraged by the absurd system of premature specialization. After the age of fourteen or fifteen, a man or woman in this country need never again come up against a serious mathematical concept. This is, surely, a gravely disturbing phenomenon, made more so by the contrast with the far more broadly-based educational requirements pursued in France, in Japan, in the Soviet Union. The damage is many-sided. Let me stress only one aspect. Those whose mathematical awareness has ceased with O-levels are, today, fairly well cut off from any but the most second-hand, mendaciously predigested view of much of modern philosophy, of modern economics, of the forms of statistical argument which penetrate more and more of our politics, psychology and social thought. They have only a minimal access to the rapid developments in current genetics and biology. They are cut off completely from anything but Sunday-newspaper physics or cosmology. Much of what is most vital in the modern condition of mind and of sensibility is wholly inaccessible to those who last drew a tangent or extracted an algebraic root in some loathed school-room in early adolescence. Tomorrow, the status of the mathematical illiterate may be worse. Computers, data-banks, retrieval systems of every description will probably bring on changes in daily economic and social life fully comparable to those caused by the first industrial revolution. A certain level of mathematical understanding, a certain unforced intimacy with the notion of algebraic series, of scatter, of fields, of classes, will be the key to this new world. Is anyone really educated who has not had mathematical instruction at least to the point where he leaves secondary schooling?

Of course there are obstacles, foremost among them the universities with their acceptance formulas based on an absurdly narrow set of A-levels, with their unwillingness—until very recently—to allow a man to continue working on parallel or interdisciplinary fronts. But there is also, I feel, widespread doubt as to the "teachability" of mathematics to those who seem recalcitrant or who plead some inherent inadequacy in their own intellectual equipment. Here there is clearly a very complex mixture of cultural, psychological and social factors. All I am able to voice is the conviction that mathematics could be taught well and taught longer to every normal schoolboy or schoolgirl if it was conceived of and presented as one of the very great languages of man. This, of course, can be meant in a technical way. Different periods in this history of mathematics, different individual mathematicians have their recognizable style and "dialect" (a page in a paper by Hilbert or Poincaré is, stylistically, visually, as unmistakable to a trained eye as would be, say, a page in a Bach score). But I intend "language" in the widest sense: mathematics, like verbal speech, is an organic, historically formed, way of perceiving, of stating and of making predictions about reality—either external reality or the no less substantive reality of thought. It is a way, incomparably subtle, coherent and often witty, of recording, elucidating and translating experience. Verbal speech, music and mathematics are very likely the three principal modes of utterance of human civilization, the three signal codes of the mind when it is most fully in play (as distinct from the more "bodily" communicative codes of gesture and the plastic arts). Seen as a major way of "speaking about", of "declaring" our relations to the world, mathematics cannot be relegated to the status of a rebarbative speciality to be dropped from the curriculum with indecent haste. It must be viewed as an integral element of human articulation, to be mastered as one masters one's native speech, at least to a certain modest level of fluency.

A good many heuristic devices follow on such a view. Concepts and problems can be taught genetically, according to the often dramatic logic of their actual historical development. The history of the liberation of space from visual, tactile limitations related Euclid to Descartes, Descartes to Riemann. The long struggle to prove that every integer can be represented as a finite product of prime numbers in which some factors may repeat themselves and that the representation is unique, leads from the ninth book of Euclid's *Elements* in 300 BC to Gauss's *Disquisitiones arithmeticae* of 1801. Why did this fundamental theorem elude the best mathematicians for 2,100 years? Introduced to the problem in its historical setting, the student will not only pick up some very handsome arithmetic but a degree of insight, difficult to structure as lucidly in any other discipline, into the growth and limitations of the life of the mind in western culture. Why not introduce a schoolchild to the notion of function as it actually evolved from the first years of the seventeenth century, to the eighteenth-century understanding of trigonometric functions and the nineteenth-century realization of boundary values? Again, the history is one of intellectual adventure, temporary defeat and the forward leap of tensed imaginative energies, and it is a history rich in general implications. Wherever possible, indeed, mathematics ought to be seen and taught as part of the structure of the relevant period, with its correlates in art, music, philosophy and the social climate of the age. The deep, genuine parallels with the best teaching of language and of literature should be evident. It follows also that those who teach mathematics to schoolchildren should be among the most highly trained, generally literate and imaginatively excited in our community. Their prestige and financial status should fully match these exacting needs. This is the case in Russia, in the best German and Swiss *Gymnasia*, in the *grands lycées* of France. Is it the case in England? How can a discipline prosper when so many who teach it to human beings at the crucial stage of mental development feel themselves to be among the defeated?

It would be impertinent for a layman to say more. And it has all been said so very much better by a young man not yet twenty-one who in the last night of his life established the basis of modern algebraic theory and topology. Galois's famous letter-memoir of May 29, 1832, concentrates every aspect of the passionate stress and adventure of mathematics. There is the exultation, so characteristic of mathematical feeling, at the fact that the imagination can glimpse a great field about which "at present we know nothing". There is the grim note of individual limitation, familiar even to those mathematicians who have known a full career: "I have not time enough and my ideas about this immense terrain are not yet well developed." There is, above all else, the possession of a living being by an energy, by a delight of pure thought and rigour so intense as to overcome even the proximity of death. How diminished is a literacy which cannot include such a text among its classics.

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Cores of the Terrestrial Planets

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The compositions of the cores of the terrestrial planets have been re-examined following a calculation by O. G. Soroktin that the iron oxide Fe_2O is stable at pressures reached in the Earth's core and his suggestion that the outer core may consist of Fe_2O. It is shown that the idea of an Fe_2O outer core in the Earth can be fairly well reconciled with a common overall composition for the planets Earth, Venus and Mars in a way that avoids the main objections to the earlier phase-transition theory.

THE presently favoured theory that the Earth's outer core consists predominantly of iron (possibly alloyed) requires[1,2] the overall compositions of the planets Earth, Venus and Mars to be markedly different. It is difficult to reconcile this result with accretion theories of the origin of the planets[1-3].

In an early attempt at reconciliation, Ramsey[4] and myself[5] independently advanced a "phase-transition theory" which treated the Earth's outer core as a high-pressure modification of the lower mantle material. This theory agreed with a common overall composition for the three planets and fitted well the then available observational data on the masses and radii of Venus and Mars and the polar flattening of Mars. The theory later met with difficulties.

Revisions of the planetary observational data reduced the quality of the original fit. The most serious surviving discrepancy is that the preferred observational estimate[6] of the radius of Venus is now 6,052 ± 10 km, as against 6,270 km predicted[7] on the phase-transition theory.

The original Earth models *A* and *B* (refs 8, 9) used in deriving models of the other planets have had to be amended (see refs 10, 11) to allow (among other things) for the reduction of the estimated Earth's central density[12] from about 18 to 13 g cm^{-3}, and of the estimated moment of inertia coefficient[13] for the Earth from 0.3336 to 0.3309. Both revisions have slightly worsened the fit with Venus and Mars.

Geochemists have found difficulty, principally on the question of the packing of oxygen atoms in the lower mantle material, in reconciling the proposed phase transition with the large density jump (in the ratio 0.7 or more) at the Earth's mantle-core boundary, *N* say.

Transitory shock-wave experiments carried out in laboratories at high pressures have failed to supply positive evidence that a phase transition occurs at *N*.

Alternative Model

Having regard to various uncertainties and questions of experimental interpretation, I think it is unsound to assert, as do some investigators, that the difficulties have "disproved" the theory—indeed the theory still has some notable followers. But the case against it is sufficiently strong to make it desirable to seek alternatives.

As a possible alternative, I propose a theory which avoids the principal difficulties of the phase-transition theory while retaining the important feature that the pressure p_c at *N* is critically involved in the changes of property at *N*. The theory incorporates a suggestion of O. E. Soroktin (personal communication) that the Earth's outer core consists of Fe_2O. Soroktin has calculated that this oxide, which is unstable at ordinary pressures, becomes stable at the pressures in the Earth's core and has a density-pressure relation matching that in the Earth's outer core.

In other respects, I have deviated substantially from Soroktin. Whereas he attributes the occurrence of Fe_2O in the outer core to a breakdown of FeO into Fe_2O and oxygen, I have associated the occurrence of the Fe_2O with the equation $Fe_2O \rightleftharpoons FeO + Fe$, my reason being the extent of planetary fit that can be thereby achieved.

My proposal, which I refer to as the "Fe_2O theory", envisages a model family of planets with the following properties:

All planets of the family are composed of two primary materials—a basic mantle material *X*, and Fe_2O. (This is subject to qualification below.) For present purposes, the composition of *X* need not be specified, but *X* is likely to include some FeO.

In all the planets, the ratio of the mass of *X* to the mass *M* of the planet is the same.

In those planets which contain Fe_2O, the Fe_2O occurs as a distinct zone (the "outer" core) throughout which $p \geqslant p_c$, where *p* is the pressure.

In those planets where the first two properties would entail $p < p_c$ in an Fe_2O zone, some or all of the Fe_2O has broken down into FeO and Fe. This FeO, which I refer to as *Y*, forms part of the mantle and is additional to any FeO that may be part of *X*. The Fe falls to form an "inner" core.

Details of the Model

The second property secures a common overall composition for the family. The masses of *Y* and Fe in any planet are such as would combine precisely to form Fe_2O when $p \geqslant p_c$, and the ratio of the total mass of Fe, Fe_2O and *Y* to *M* is the same for the whole family.

The family has three subsets—*H*, *J*, *K*, say (Fig. 1). The subset *H* includes the smallest planets which have no Fe_2O zones and thus no outer cores; they have mantles composed of *X* and *Y* mixed, and inner cores of Fe. The subset *K* includes the largest planets which have mantles composed purely of *X*, outer cores of Fe_2O, and no inner cores. The subset *J* consists of intermediate planets which have mantles composed of *X* and some *Y*, outer cores of Fe_2O, and inner cores of Fe. For all the *J* planets, $p = p_c$ at the top of the outer core. The core-mantle mass ratios are the same for all *H* planets, and for all *K* planets (but the ratio is different for *H* and *K*).

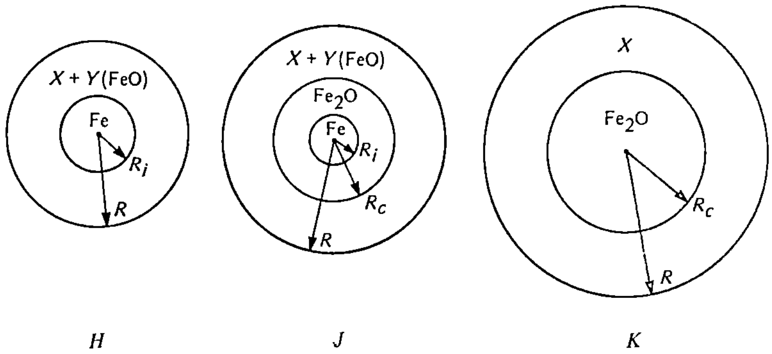

Fig. 1 Materials in the interiors of the three subsets (*H*, *J*, *K*) of terrestrial planets. The outermost zones, all containing the material *X*, are mantles. The Fe_2O zones are referred to as "outer cores", the Fe zones as "inner cores".

Earth and Venus would correspond to members of *J*, Mars of *H*. Unfortunately, no known planets correspond to *K*, so that an observational test of the subset *K* is not available. From evidence on the Earth, all Fe_2O zones (outer cores) would be expected to be fluid, and Fe zones (inner cores) solid.

The Fe_2O theory resembles the phase-transition theory in that all *J* planets have the same (critical) pressure p_c at the mantle-core boundaries, and all *H* planets lack outer cores. To a considerable extent, it is this feature which enables both theories to go close to fitting a common overall composition for Earth, Venus and Mars. Differences in detail arise because, in planets smaller than Earth, the Fe_2O theory entails larger iron (inner) cores than does the phase-transition theory.

Agreement with Observation

I have started from a simplified Earth model, taken to be a member of the subset *J*, in which the mean densities of the mantle, outer and inner cores are 4.5, 11 and 13 g cm^{-3}, respectively. I have provisionally neglected variations of density inside planetary zones and taken the ratio of the mean densities of *X* and *Y* as 0.7. For a first approximation, differences of compression between corresponding zones of the *J* planets have also been neglected. Possible temperature differences between different planetary interiors, volume changes due to chemical interaction, and so on, in the mixing of *X* and *Y* in mantles, and the presence of nickel in cores have been neglected as likely to affect the essential numerical detail only slightly.

With these simplifications, the postulates formally determine numerical details for all the *J* planets. In particular, a set of values of *M*, *R* (radius of the planet), R_c (radius of the outer core) and R_i (radius of the inner core) is determined.

The observational mass of Venus[14] is 4.87×10^{24} kg. For the *J* planet with this mass, the simplified Fe_2O theory gives $R=6{,}010$ km, $R_c-2{,}710$ km, $R_i=2{,}110$ km. Allowance for the neglected compression differences will increase this estimate of *R* because Venus, being smaller, is less compressed than the Earth. Thus the Fe_2O theory fits the observed radius ($\sim$6,050 km) of Venus as well as can be expected.

The value 600 km yielded for the thickness, *T* say, of the (presumed fluid) Venus outer core is also interesting. It may be contrasted with the value exceeding 900 km (ref. 7) on the phase-transition theory and substantially larger values on theories which assume a predominantly iron outer core. By yielding the lowest value of *T*, the Fe_2O theory accounts best for the failure to observe a significant magnetic field around Venus; the presumed seat of the Earth's magnetic field (the Earth's outer core) is 2,200 km thick.

The postulates also determine the ratio of the mass of the inner core to the mass *M* of any *H* planet as approximately 0.15. For a particular *H* planet of assigned mass, a first approximation to *R* is then formally yielded if values are available for the mean densities, ρ_1 and ρ_2 say, of the core and mantle. For Mars, $M=0.642 \times 10^{24}$ kg. For the *H* planet with this value of *M*, taking ρ_1, $\rho_2=9.0$, 3.7 g cm^{-3}, the simplified Fe_2O theory gives $R=3{,}354$ km; the yielded value of *R* is increased by 10 km if the trial value of ρ_1 is decreased by about 1.0 g cm^{-3}, or of ρ_2 by about 0.03 g cm^{-3}. Thus uncertainties in the appropriate values of ρ_1 and ρ_2 could possibly permit raising *R* to about 3,370 km, which is, however, less than the presently preferred observational value[3,15,16] of $3{,}388 \pm 5$ km.

When hydrostatic conditions are assumed throughout Mars and ρ_1 and ρ_2 are treated as constant, the Fe_2O theory yields $f=1/191$ and $\delta=18$ km, where *f* and δ are, respectively, the polar flattening and the excess of the equatorial over the polar radius. These results agree closely with the observed *f* and δ (see refs 3, 17). But allowance for density variation inside the mantle is likely to lower the calculated values to 1/200 (or even less) and 17 km. These last values are on the border[17] of the limits of error of the hydrostatic theory.

The phase-transition theory gives $R=3{,}390$ km and $f \approx 1/188$ (ref. 18), assuming hydrostatic conditions. Thus the phase-transition theory gives the better fit with Mars unless the departures from hydrostatic conditions are considerable. This is principally because the Fe_2O theory requires a larger core, radius $R_i \sim 1{,}400$ km. It is likely that seismic records taken on Mars will in due course provide an observational estimate of R_i and so serve as a further test of the theories. (The gathering of seismic records on Venus is likely to be more difficult.)

Ironically, after the phase-transition theory had been shown to fit closely the then assumed observational estimate $R=3{,}390$ km for Mars, several investigators were disposed to reject that theory by insisting on observational values of 3,350 km or less[17]. A value of 3,350 km would fit the Fe_2O theory extremely well.

Assuming, as usual, that the mantle and iron core of Mars are solid (ref. 19) the Fe_2O theory, in common with the phase-transition theory, accounts satisfactorily for the failure to detect a magnetic field around Mars.

So the provisional calculations indicate that the Fe_2O theory fits the observational *M* and *R* for Venus extremely well, and *M*, *R* and *f* for Mars moderately well. The theory also gives the closest agreement of all theories with the observational evidence on magnetic fields.

Like all theories that are not extremely *ad hoc*, the Fe_2O theory does not fit the planet Mercury as Mercury now is, although the theory has an advantage over the phase-transition theory in requiring a substantially larger iron core. As I have previously suggested[20], Mercury, through its proximity to the Sun, has probably lost much of its primordial mantle by volatilization. The Fe_2O theory would suggest that the primordial Mercury (assumed to belong to the subset *H*) was larger than Mars.

The low density of the Moon precludes it from belonging to the family of planets considered here. The *H* planet with the Moon's mass would have an iron core of radius about 700 km, which is appreciably too large to fit evidence on the Moon's mean density and moment of inertia. Thus the Fe_2O theory here merely confirms that the Moon must have had an exceptional origin.

I hope to present later calculations which take detailed account of compression and density variation inside the planetary zones.

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Skull of *Hesperornis* and Early Evolution of Birds

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Additional preparation of a specimen found a century ago permits complete reconstruction of the skull of the Cretaceous bird *Hesperornis*. This skull provides new evidence on the evolution of the avian palate and the origin of avian cranial kinesis, and further substantiates the evolution of birds from theropod dinosaurs.

IN spite of some disagreement about the immediate ancestry of birds[1–3], there is little doubt that the Late Jurassic *Archaeopteryx lithographica* is the earliest known representative of the class Aves. *Archaeopteryx* was a very primitive reptile-like bird—as Ostrom[3] states, the *Archaeopteryx* specimens would probably have been identified as dinosaurs if feather impressions had not been preserved with them. By the end of the Cretaceous, about 70 m.y. after *Archaeopteryx*, the avian fauna apparently consisted exclusively of birds of modern aspect referable to Tertiary orders[4]. The only well preserved specimens intermediate in age and morphology between the dinosaur-like *Archaeopteryx* and modern birds are two genera, *Hesperornis* and *Ichthyornis*, described by O. C. Marsh a century ago from rocks of early Late Cretaceous age.

During Marsh's expedition in 1871 to the marine Niobrara Chalk of western Kansas, he collected much of the post-cranial skeleton of a large swimming bird, which he named *Hesperornis regalis*[5]. Shortly thereafter a complete skeleton of *H. regalis* (Yale Peabody Museum 1206) was found. The skull of this specimen included the crushed braincase and pieces of the upper and lower jaws. Many of these bones were cleaned on one side only and never removed from the original matrix. The braincase and other cranial fragments of YPM 1206, together with a broken but uncrushed occipital segment of another braincase (YPM 1207), formed the basis for the discussion and reconstruction of the skull of *Hesperornis* given by Marsh in his monograph on the Odontornithes[6], or toothed birds. Marsh's skeletal reconstruction shows *Hesperornis* to have been a flightless, predaceous bird, probably similar in habits and diet to the living loons.

Following the publication of Marsh's monograph, Lucas[7] described another partial skull of *Hesperornis regalis*, including the premaxillae and parts of the mandibles (US National Museum 4978), and a nearly complete but crushed skull of *Hesperornis gracilis* (University of Kansas 2287). *H. gracilis* differs from *H. regalis* only in being slightly smaller.

Marsh stated in his monograph[6] that "in its more important characters, the skull of *Hesperornis* resembles that of the Ratitae, or Struthious birds". This view, as is discussed later, was immediately challenged by D'Arcy Thompson, and further discredited by Lucas, Shufeldt and Heilmann, none of whom restudied Marsh's original material. A separate, more recent controversy concerns the presence of teeth in the skulls of *Ichthyornis*[8] and *Hesperornis*[9]. A new partial mandible of *Ichthyornis* and restudy of the best preserved Yale skull of *Hesperornis* (YPM 1206) has confirmed Marsh's descriptions. The jaws of both *Ichthyornis* and *Hesperornis* bore true thecodont dentitions[10].

The retention of thecodont teeth in birds that appear in many other features to be fully modern is evidence that *Hesperornis* and *Ichthyornis* are truly intermediate between *Archaeopteryx* and modern birds. *Hesperornis*, being represented by parts of four skulls, is thus of great importance in evaluating several problems of early avian evolution, including the early evolution of the palate and the origin of avian cranial kinesis. The origin and function of the intramandibular articulation of *Hesperornis* is also discussed later.

Structure of the Palate

The new reconstruction of the skull of *Hesperornis regalis*, shown in Fig. 1, incorporates the information gained

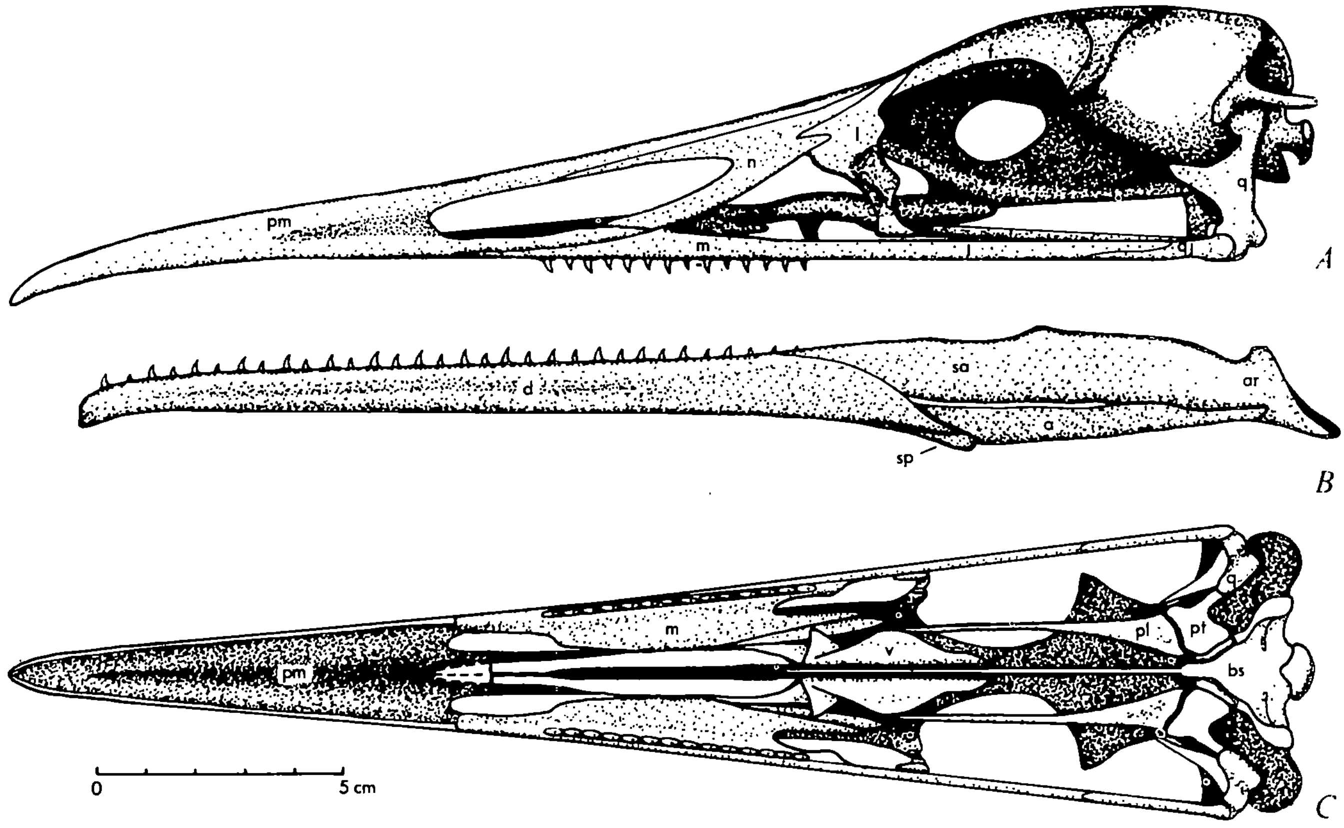

Fig. 1 New reconstruction of the skull of *Hesperornis regalis*. Cranium (*A*) and mandible (*B*) in lateral view, palate (*C*) in ventral view. a, Angular; ar, articular; bs, basisphenoid; d, dentary; f, frontal; j, jugal; l, lacrimal; m, maxilla; n, nasal; pl, palatine; pm, premaxilla; pt, pterygoid; q, quadrate; sa, surangular; sp, splenial; v, vomer.

from further preparation of the skull of YPM 1206, and from detailed comparison of it with the other three known skulls of *Hesperornis*. One of the most important discoveries resulting from preparation of YPM 1206 is the true identity of several of the bones of the palate. The bone illustrated by Marsh as a vomer (ref. 6, Plate 2, Fig. 8) is in fact an almost complete left palatine, preserving articular facets for both the pterygoid and the vomer. The bone identified in Marsh's Plate 2, Fig. 9 as a palatine is actually the right vomer.

The corrected relationship of the bones of the palate of *Hesperornis* is illustrated in Fig. 1*C*. The pterygoid is a relatively small bone with a concave articular surface contacting the quadrate just above its medial keel. The medial process of the pterygoid terminates in a large facet which articulated with a short, broad "basipterygoid" process of the basisphenoid. The pterygoid and palatine were joined along a relatively immovable joint. The palatine is broad at its posterior end, and tapers anteriorly. It is fused anteriorly to the dorsolateral surface of the posterior part of the vomer. The vomers are long thin bones which are expanded posteriorly in association with the openings of the internal nares. They articulated with a narrow parasphenoid rostrum posteriorly, and apparently formed an anterior extension of it. The vomers were separate for all of their preserved length but the anterior ends are missing and may have been fused. It is not possible to determine exactly the original conformation of the expanded posterior end of the vomers, again because of breakage. The vomers certainly did not approach contact with the pterygoids. Approximately the medial third of each vomer was sutured to the corresponding maxilla.

All the palatal bones are now disarticulated but, considering the morphology of the articular surfaces, it is unlikely that significant relative movement was possible within the palatine-vomer-maxilla complex. The palatine and pterygoid were firmly joined, though not fused. The articular surfaces joining the quadrate and pterygoid, and the pterygoid and basisphenoid are smooth and some movement between these elements no doubt took place.

Huxley, in his 1867 classification[11], separated the ratites (in his opinion a primitive group) from other birds, citing their unkeeled sternum, lack of a sphenoid rostrum-palatine articulation, strong "basipterygoid" processes arising from the basisphenoid, undivided proximal head of the quadrate and so forth. Marsh[6] based his statement of the ratite affinities of *Hesperornis* chiefly on these four characters. D'Arcy Thompson[12] challenged Marsh's interpretation, and from a study of Marsh's figures concluded that *Hesperornis* was related most closely to the living loons and grebes. Lucas[7] supplemented Thompson's list of differences between *Hesperornis* and the ratites. Finally, Shufeldt[13] published a reconstruction of the palate of *Hesperornis* based on Marsh's published figures, several poor photographs of the specimens, and several skulls of loons—not surprisingly, his reconstructed palate closely resembles the palate of a loon. Little was left for Heilmann[14] to do in his classic book on the origin of birds, but to quote Shufeldt[13]: "It will once and for all disabuse the mind of the paleontologist with respect to there being any agreement of characters when we come to compare the skull of the ostrich with that part of the skeleton of *Hesperornis*". As Marsh's specimens were never restudied, this view has gone unchallenged.

More recently De Beer[15] reasoned that because the plumage and the lack of fusion of the bones of the braincase in ratites are derived by neoteny, and as the palate of *Archaeopteryx* was neognathous (based on the reconstruction of Kleinschmidt[16]), it is probable that the "palaeognathous" palate of the living ratites is derived by neoteny from the "neognathous" type characteristic of the majority of living birds. Unfortunately Kleinschmidt's reconstruction of the palate of *Archaeopteryx* is completely hypothetical—the morphology of the palate cannot be determined in the known specimens. In spite of this, De Beer's idea that the palaeognathous avian palate is derived from a neognathous ancestor has since been elaborated[17], and has apparently gained wide acceptance[18].

The structure of the palate of *Hesperornis* is obviously of primary importance in considering the evolution of the avian palate. When Pycraft[19] originally proposed a fundamental dichotomy between the palaeognathous and the neognathous birds, he stated that the skull of the Palaeognathae differs from all other known birds in having a firm, immovable pterygoid-palatine connexion. Huxley's diagnosis[11] of the dromaeognathous palate (=palaeognathous palate of Pycraft) based on the absence of a palatine-sphenoid contact and on the presence of basipterygoid processes arising from the body of the basisphenoid, also distinguishes the palaeognathous palate from that of neognathous birds.

The palate of *Hesperornis* (Fig. 1C) was clearly palaeognathous in all diagnostic features. The pterygoid and palatine were firmly joined. The palatines were separated from the sphenoid rostrum by the pterygoids, and strong basipterygoid processes (which articulated with the pterygoids) were present. Considering the morphology of the palate of *Hesperornis*, the palaeognathous condition is probably truly primitive in birds. This does not, of course, imply that the ratites are descended from flightless ancestors as some early writers maintained. There is good anatomical evidence that ratites evolved from ancestors capable of flight[15].

Recent studies on the egg white proteins of ratites[20,21] indicate that the large ratites (*Struthio, Casuarius, Dromaius* and *Rhea*) are more closely related to one another than to any other group of living birds, supporting classification of them together in the order Struthioniformes. The kiwis (*Apteryx*) and the tinamous apparently constitute two separate groups, and are retained in the orders Apterygiformes and Tinamiformes respectively. These three living groups of palaeognathous birds seem to represent three surviving orders of the first major radiation of birds. Most of this Cretaceous radiation (which included *Hesperornis*) is now extinct. To quote Huxley[11]: "Hence, in all probability, the existing Ratitae are but the waifs and strays of what was once a very large and important group."

Intracranial Mobility

Most birds in addition to raising and lowering the lower bill, are able to raise and lower the upper bill relative to the braincase. The general function of this cranial "kinesis" presents one of the most interesting unsolved problems of avian skull morphology. Simonetta[22] and Bock[23] have published important reviews of the kinetic movement of birds, and Frazzetta[24] has reviewed the cranial kinesis of lizards. Raising the upper bill by bending the nasals and premaxillae (as in living palaeognathous birds) is termed rhynchokinesis. Raising the upper bill about a transverse hinge between the frontals and premaxillae (as in neognathous birds) is termed prokinesis. Mesokinesis is the condition in some reptiles where the upper jaw is raised by means of a transverse hinge between the frontal and parietal bones.

In *Hesperornis* the premaxillae and nasals overlapped the frontals by about 1.5 cm. The premaxillae and nasals appear to have been firmly sutured to the frontals, ruling out a prokinetic hinge at this point. The premaxillae and nasals were joined above and below the external nares, forming a single rigid member and eliminating rhynchokinesis. The quadrates, however, appear to have been moveable (streptostylic), a characteristic which in modern birds is associated with some kind of kinetic mobility. The articulation of the maxillae with the nasals and premaxillae reveals how streptostyly was associated with an akinetic skull in *Hesperornis*.

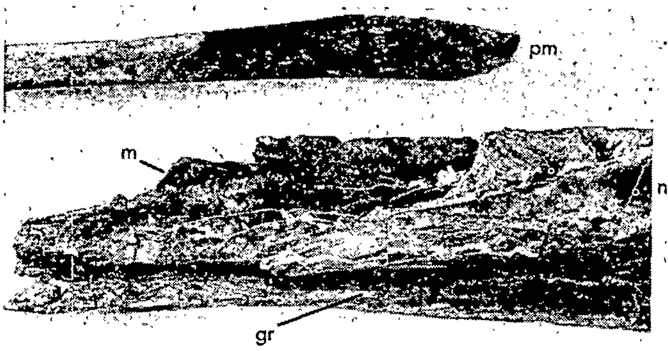

Fig. 2 Dorsal view of left maxilla (m) of *Hesperornis regalis*, YPM 1206, showing groove (gr) which fits over nasal-premaxilla bar. Nasal (n) preserved against maxilla. Nasal and premaxilla (pm) in lateral view, dashed line shows degree of overlap of premaxilla on nasal.

In the Yale skull of *Hesperornis* (YPM 1206) the fused premaxillae, the left and right nasals, and the left and right maxillae are all disarticulated, permitting examination of all articular surfaces. Rod-like ventral processes of the premaxillae and nasals were joined by a long overlapping suture below the external nares (Fig. 2). Together these formed a smooth subnarial bar on each side of the skull. A smooth groove is present above the tooth row on the dorsal surface of each maxilla (Fig. 2). The corresponding subnarial bar clearly fits into this groove. The smooth surfaces of the maxillary grooves and subnarial bars indicate that they were not firmly joined but articulated by a sliding mechanism, with the subnarial bars of the nasals and premaxillae forming rails to guide forward and backward movements of the maxillae.

Further evidence that the maxillae articulated freely with the nasals and premaxillae is provided by the degree of disarticulation of the skull of *Hesperornis gracilis* (UK 2287). This skull is crushed, and is now preserved palate upward on a slab of chalk. The subnarial bars formed by the nasals and premaxillae have remained essentially intact on both sides of the palate. The maxillae have both separated cleanly from the subnarial bars, and they are either crushed into the centre of the palate or missing. It seems unlikely that both maxillae would be lost from an otherwise articulated skull if they had been firmly joined to it.

Thus, streptostyly in *Hesperornis* apparently was associated only with fore-aft movement of the maxillae relative to the rest of the skull, and not with prokinesis or rhynchokinesis as it is in modern birds. The exact distance that the maxillae were able to slide relative to the rest of the cranium cannot be determined accurately, but it seems to have been approximately equal to or slightly greater than the distance between two adjacent teeth, that is, about 2 to 3 mm. The function of this sliding mechanism is not clear. It possibly assisted in pulling prey into the mouth, or in disengaging the maxillary teeth from prey. In this regard it is significant that the upper teeth occur only in the sliding maxillary segments. The premaxillary part of the upper bill was edentulous and apparently covered with a horny bill similar to that of living birds.

The absence of a standard form of kinesis in *Hesperornis*, a primitive bird because of its geological age and its retention of thecodont teeth, requires a new interpretation of the evolution of kinesis in birds. In his review of avian kinesis Bock[23] attempted, as Versluys[25] had previously, to trace kinesis continuously from a kinetic reptilian ancestor to birds. Bock reasoned that because none of the biological roles generally ascribed to avian kinesis imparts any obvious advantage, kinesis was probably inherited from reptilian ancestors rather than developed independently in birds. Following this reasoning, Bock interpreted the poorly preserved skull of *Archaeopteryx* as mesokinetic.

Two recent developments argue against this. As described earlier, the skull of *Hesperornis* was akinetic. Moreover, Ostrom's[3] evidence of direct phyletic relationship between *Archaeopteryx* and coelurosaurs indicates that birds evolved from this group of theropod dinosaurs. It has not been convincingly demonstrated that any theropod possessed a kinetic skull. Colbert and Russell[26] interpreted the skull of *Dromaeosaurus albertensis* as mesokinetic, but the supposed mesokinetic hinge is not preserved in the only known skull of this species. Furthermore, the quadrate and pterygoid on each side of the skull of *Dromaeosaurus* have broad, thin, overlapping processes which appear to have been joined together tightly. The suggestion that the skull of *Deinonychus* may have been kinetic[27] was based only on the disarticulated nature of the preserved specimens. Restudy of *Dromaeosaurus* and *Deinonychus* has provided no evidence that either had a kinetic skull. Russell[28] demonstrated that the skulls of the large theropods (tyrannosaurs) were akinetic. Consequently it seems very unlikely that birds inherited any form of kinesis from their reptilian ancestors. Even if theropods and *Archeopteryx* had mesokinetic skulls, this would not explain the origin of avian rhynchokinesis and prokinesis. Cranial kinesis in birds seems to have had an independent evolutionary origin.

Unfortunately the general function of avian kinesis is still not well understood. The discovery that the toothed bird *Hesperornis* was akinetic suggests a new explanation of the function of kinesis in birds. Teeth such as those of *Hesperornis* are useful for holding, and for puncturing and ripping prey. The mandibles of *Hesperornis* articulated with the quadrates by a sliding joint permitting fore and aft motion of the mandibles relative to the cranium. By coordinated movement of the mandibles and maxillae food could be advanced into the mouth. The function of the bill in most birds lacking teeth must be to slice rather than to puncture. The slicing ability of the upper and lower bills of modern birds is related not only to the sharpness of their edges, but also to their angle of relative movement. As the lower beak moves almost parallel to the upper, they form an efficient slicing device. It is probable that the conventional forms of avian kinesis evolved after the loss of teeth and the development of a slicing horny bill.

Kinesis increases the amount of possible movement of the lower bill relative to the upper. In the pheasant *Phasianus colchicus* (of skull length 73 mm) the total possible fore-aft motion of the articular bone relative to the quadrate is 2 mm. Thus, if the pheasant skull was akinetic, the fore-aft motion of the lower bill relative to the upper bill would be limited to 2 mm. The skull, however, is kinetic. If the upper bill is depressed when the lower bill is protruded, and then raised by pressure of the lower bill as it is adducted, the lower bill moves approximately 6.5 mm relative to the upper (determined by manipulation of a freshly killed specimen). This is more than a three-fold increase in the relative movement of the two elements. Thus a kinetic skull greatly increases the relative movement of the upper and lower bills, and so significantly augments shearing with the beak. Both the mandibular adductor and the pterygoideus muscles in most birds are aligned with the shearing region of the beak, suggesting that the beak can be used efficiently for shearing during both adduction and protrusion of the lower bill[29].

The evolution of prokinesis and rhynchokinesis from an ancestor with a skull similar to *Hesperornis* involved refusion of the maxillae to the premaxillae and nasals, and either the development of a line of bending between the frontals and the premaxilla-nasal rostrum, or the reduction and loss of the postnarial connexion between the nasals and maxillae. Prokinesis and rhynchokinesis may have evolved independently in neognathous and palaeognathous birds, or rhynchokinesis may have evolved from some form of prokinesis. The exact evolutionary sequence cannot be determined without additional well preserved skulls of fossil birds.

Intramandibular Articulation

One additional feature of *Hesperornis* should be mentioned. Each mandible of *Hesperornis* is composed of six bones arranged in approximately the same configuration as those of most theropod dinosaurs. The external mandibular fenestra lies above the angular and below the surangular (Fig. 1*B*) as is typical of reptiles. Thus Cracraft's[30] speculation about the avian rather than reptilian position of this fenestra in the poorly preserved mandible of *Archaeopteryx* is probably incorrect.

There is an articulation between the splenial and angular in each mandible of *Hesperornis* (and *Ichthyornis*). Gregory[31] drew attention to this intramandibular joint and to a similar one which apparently evolved independently in mosasaurs. It has not been noted previously that the mandibles of many theropod dinosaurs (such as *Deinonychus* and *Dromaeosaurus*) have an intramandibular articulation identical to that of *Hesperornis*. The intramandibular articulation in *Hesperornis* may thus be an inherited character rather than one which evolved independently, as Gregory thought, in connexion with fish predation. The function of the intramandibular articulation in *Hesperornis* and theropods was probably to permit medial rotation of the posterior segment of the mandible independently of the dentary, thus facilitating disengaging the lower teeth from prey as the mandible was protruded. The presence of an intramandibular articulation in *Hesperornis* seems to be an important additional character linking early birds to a theropod ancestry. Unfortunately the mandible of *Archaeopteryx* is too badly damaged to determine if an intramandibular articulation was present in that form.

I have profited greatly throughout this study from discussions with Professor J. H. Ostrom, Yale University. Others who have assisted will be acknowledged in a more detailed paper, in preparation, on the skull of *Hesperornis*.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Tidal Tilt Measurement in Europe

For many years instruments have been installed in underground observatories throughout Europe and elsewhere to observe the tilting of the Earth's surface caused by Earth tides and the loading effects of the ocean and shallow-sea tides. Analysis methods have been developed to permit comparison between the measured data and theoretical tides generated from astronomical data and information concerning Earth rigidity and the distribution of tidal waters. But the agreement between theoretical and observed tides has been poor and is often inconsistent between adjacent observatories, especially in the relatively dense European network.

In some cases where several instruments are installed in different parts of the same observatory[1] discrepancies between identical instruments have been observed. The discrepancies consist of both variations in amplitude and phase of the observed tidal harmonics and although it is possible to account for incorrect calibration as a source of amplitude errors, phase differences are not to be expected from the instruments themselves.

We discuss here a possible way by which amplitude and phase errors occur in tilt measurement, and we introduce the concept of "site generated tilting" to account for the tilt inconsistencies found within underground observatories.

The tidal magnitude at a site may be described using a spectral function $\boldsymbol{H}_T(f)$ which is due to the local site tilt $\boldsymbol{S}_T(f)$, the tilt due to the Earth body tide $\boldsymbol{G}_T(f)$ and the ocean and sea loading tide $\boldsymbol{L}_T(f)$ such that

$$\boldsymbol{H}_T(f) = \boldsymbol{S}_T(f) + \boldsymbol{G}_T(f) + \boldsymbol{L}_T(f) \tag{1}$$

The functions are complex, having both amplitude and phase. Thus the addition is a vector addition.

The differences between the observed and theoretical tilts determined where no allowance is made for the site effects are quoted by Lennon and Baker[2] to be ±40% in magnitude for the North–South M_2 tilt and ±15% for the East–West M_2 tilt. Phase errors of ±10% are present in both.

The site effect vector necessary to cause these effects therefore must be of the same order of magnitude as both the body-Earth tilt and the sea and ocean load tilts themselves and it must in general differ from both the other effects in phase. It is not possible to attribute the observed phase anomalies to the instruments. Also, no likely site structure will affect the phase of local tilting and it is difficult to think of a plausible structure which will significantly affect the amplitude.

It has been suggested that anomalies in the diurnal O_1 and K_1 tides are due to daily climatic effects. Our experience with strainmeter measurements suggests that the thermal noise is small in reasonable sites. In any case, in the frequency transform, climatic effects can be clearly distinguished from tidal peaks.

The only source of local tilting with the right sort of spectrum is to be found in the rock stresses set up by tides. These stresses are determined by the tidal strain field. Where inhomogeneities of rock properties occur, local anomalies in the strain fields result. Associated with these anomalies are tilts. The magnitude and phase of the tilts are dependent on the magnitude and phase of the components of the tidal strain tensor for the region and on the geometry of the rigidity inhomogeneity.

Not all the components of the body strain tide are in phase with the tidal tilt components; for example, the East–West linear strain is 90° out of phase with East–West tilt, but North–South tilts and strains are in phase. This applies to both the diurnal and semidiurnal tidal species. Phase differences may also occur for tidal tilts and strains due to tidal loading but a discussion of this would be elaborate.

As an example of the way in which tilt-strain coupling may occur we consider the tilts induced in a tunnel wall due to a linear strain field imposed at a distance from the tunnel. This problem is relevant to the manner in which pendulum type tiltmeters are sited in observatories at present.

The problem of tunnels in elastic media subject to various stress fields is covered extensively in the literature of rock mechanics. The change of radius of a tunnel in an infinite block subject to a strain in regions distant from the tunnel is given by[3]

$$\delta r = e_{xx} r_0 (1 + 2\cos 2\theta) \tag{2}$$

where r_0 is the tunnel radius when $e_{xx}=0$, and θ is an angle to the horizontal (Fig. 1*a*).

The tilting at the tunnel wall about the *y* axis is given by

$$\begin{aligned} w_y &= \frac{1}{r_0}\frac{\partial r}{\partial \theta} \\ &= -4e_{xx}\sin 2\theta \end{aligned} \tag{3}$$

At the positions marked in Fig. 1*b* strain-coupled tilts of $\pm 4e_{xx}$ occur. Thus it is possible for strain-coupled tilts with magnitudes comparable to the theoretical body and load tidal tilts to occur. For example a linear strain tide of 1 part in 10^8 could give rise to local tilting of 4×10^{-8} rad.

Thus the value of $\boldsymbol{S}_T(f)$ generated by this mechanism can take on the necessary magnitude and phase to explain the differences between the observed and theoretical values of $\boldsymbol{H}_T(f)$.

It is important to position tiltmeters so as to minimize the effects of site generated tilts. The alternative approach, that of correcting the observed tilts by calculating the magnitude and phase of the strain generated tilt has many practical difficulties, primarily the difficulty of knowing the exact geometry and rigidity distribution of the cavity walls. This approach is therefore best ignored.

Horizontal pendulum type tiltmeters are often installed in niches cut into the side of underground cavities. Clearly in the ideal case (Fig. 1*c*) tiltmeters could be installed at positions A, B, C and D. But in real underground sites even when these are of simple geometrical form, uncertainties caused by fissures in the surrounding rock make it unrealistic to determine the exact positions of minimum strain–tilt coupling.

Provided that the tunnel is horizontal and beneath a uniform ground surface, however, tilts along the axis of the tunnel will be unaffected by tidal rock stresses. Thus when tiltmeters are sited in tunnels they should be arranged to measure tilt only along the tunnel axis. Separate tunnels should be used for

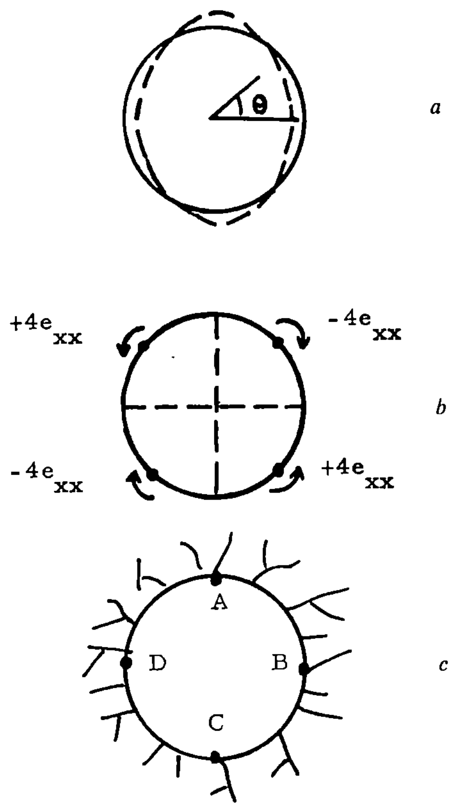

Fig. 1 Strain–tilt coupling caused by a tunnel. The *y* direction is along the tunnel.

the other components. Because of irregularities in the tunnel surface a suitable installation method might be to place the tiltmeter on a long beam on the floor of the cavity supported perhaps by sand at each end.

These comments apply only to pendulum tiltmeters sited in tunnels. They do not apply to borehole tiltmeters when actually used in a borehole, nor do they apply to water tube tiltmeters which are almost always mounted along the length of tunnels because of the length of water tube required to get adequate sensitivity.

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Tidal Tilt Anomalies

King and Bilham[1] have made an interesting suggestion for a mechanism to explain the large number of published anomalous tidal tilt results. The proposed coupling between the tidal strain and the tidal tilt leads to tilt anomalies of the order of 8 msec for the M_2 body tide and, for the loading tide, anomalies depending on the magnitude of the loading strain.

A mine or tunnel, in which tilt measurements are usually taken, represents a discontinuity in the Earth's crust[2]. The strain-tilt coupling clearly provides a possible mechanism for understanding the station cavity problem. Here we present some experimental evidence which generally agrees with the predictions of the mechanism and discuss the implications.

An intensive programme of tilt measurements has been performed in the past few years, in the vault at Bidston, chiefly as a test of instrumental performance utilizing the very large loading signal (M_2 amplitude: 70 msec north-south; 55 msec east-west) before progressing to the geophysical station at Llanrwst. A variety of different designs of tiltmeter have been used: two Verbaandert/Melchior horizontal pendulums; two Tomaschek-Schaffernicht pendulums; one smaller Tomaschek pendulum; two modified Milne-Shaw pendulums; and two Askania vertical pendulums. The vault is 15 m by 2.5 m with its length precisely in the east-west azimuth. Much data has been obtained from these different instruments at sites along the length of the vault but the general conclusions are independent of the particular instrumental design. The M_2 phase is consistent in both the north-south and east-west azimuths independent of position within the vault. Similarly, the amplitude of the east-west tilt seems to be mostly independent of position within the vault even for measurements taken at the two ends of the vault[3]. The magnitude of the north-south tilt, however, depends on position, and varies by 20% from one end of the vault to the other.

If, for simplicity, we regard the vault as a cylindrical cavity of length 15 m and diameter 2.5 m, then the results are seen to conform to the general predictions of King and Bilham. Tilts along the axis of the cylinder are more consistent than those across the cylinder. It is interesting that the north-south variations exist even though from symmetry considerations most of the measurements are taken at the points where the anomaly may be expected to be a minimum (ref. 1: Fig. 1*c*, position C), showing that the real situation is indeed more complicated than these simple considerations might suggest. In fact, the vault is excavated in the sandstone below the Bidston observatory, so the local topography and general environment are perhaps too complex to justify more definitive investigations on these lines.

Work is in progress to identify such effects in the geophysical station at Llanrwst. There are already some indications from a very short time series using the prototype Askania vertical pendulum in comparison with the Tomaschek horizontal pendulums that more consistent tilts are recorded in azimuths corresponding to the axis of the tunnel.

If these indications prove to be correct then, clearly, there are many general points to be considered regarding instrument installation. Ideally, the instrument should be as far away from the wall of the cavity as is possible, at least for measuring the tilt in the azimuth normal to the wall. The common practice of using special niches cut into the rock must be viewed with caution, especially for the azimuth out of the wall. Indeed the niche itself may respond in some unknown way. Again, a vertical pendulum may be more reliably employed in measuring the tilt parallel to the wall to which it is clamped. Although the recent rapid instrumental development of tiltmeters is welcome, the most basic problem lies in the siting of the instruments rather than in the instruments themselves.

Concentration of effort in measuring the tilt along the tunnel axis may be the best policy to adopt but, in any case[2,3], detailed measurements of the coherency of the tidal signal over a single station should be made before attempting to discuss the regional tilt signal. In a real situation any coupling effects will be extremely complicated and can only be investigated by such a detailed experimental programme. It is worth noting that if the effects are non-linear then indication of this may be obtained by examining the higher frequency components such as M_4 and MS_4. The use of special shafts or boreholes, although having their own problems, may be less affected by this coupling problem and may eventually provide an alternative solution.

King and Bilham[1] suggest that the strain-tilt coupling at a particular site is too difficult to calculate and that therefore this approach should be ignored. Nevertheless, there is much experimental information which should be reassessed in the light of this coupling, if station configuration could be ascertained. It would be interesting to consider under what circumstances the perturbation leads to a decrease of the recorded tilt as opposed to an amplification. This will presumably depend on the location of the instrument with respect to the walls of the cavity. For example, a north-south tiltmeter on a north wall will be perturbed in an opposite sense to a north-south tiltmeter on a south wall. In the Ardennes, at four different stations, anomalously low M_2 north-south tilts have been observed. Is this related to tilt/strain coupling or is there a larger scale perturbation, for example arising from the tidal effects in an aquifier as suggested by Bower[4]? One particular point of interest (G. C. P. King and R. G. Bilham, private communication) is that the mechanism may provide an explanation of the greater magnitude of the north-south M_2 diminishing factor anomalies by comparison with east-west anomalies in Europe[2,3].

In this area the amplitude of north-south tilt is about half that of east-west tilt, whereas theory suggests the opposite to be the case for strain. This, combined with the differing phase relationship between tilt and strain in the two directional components, would seem to provide a clue as to the explanation of the bias in the observed anomalies in Europe.

King and Bilham[1] state that amplitude anomalies are often attributed to poor instrument calibration; this is not usually possible because even poor instrument calibration rarely leads to an error greater than 8% and most of the observed anomalies are significantly larger than this. Also, they conclude that phase anomalies cannot be attributed to instrumental causes. This is an oversimplification of the case. Even with gravity-meters phase errors are well known. With tiltmeters phase errors can arise from the following causes[4]: ill defined true recording azimuth of a tiltmeter; errors in surveying procedures; a drifting recording azimuth (in the case of a horizontal pendulum); and time errors. These errors are often accorded insufficient attention. The general picture of phase anomalies is probably confused by the practice of transforming from the actual recording azimuth to north-south and east-west azimuths in publishing results, so that the phase anomaly becomes a function of the amplitude anomaly and the angle of rotation of the reference azimuths.

As regards meteorological or other variable influences on tilt, the best approach is to analyse a long time series (preferably greater than 1 yr) in separate monthly sections, allowing for constituent separation problems. Only in this way can the reliability of the diurnal constituents O_1 and K_1, and indeed other constituents, be assessed accurately for any given site.

We conclude that it now seems worthwhile to examine the present experimental evidence, in the light of the proposed mechanism, to see if a consistent pattern emerges. From the experience so gained and from further detailed experimental investigation consistent and geophysically useful tidal tilt information should ensue.

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Geochemical and Geophysical Implications of the Reaction between Seawater and the Oceanic Crust

I have presented a detailed synthesis of the evidence for large scale chemical reactions between seawater and the upper 2–3 km of the ocean floor basalt[1]. These reactions seem reasonable as the ocean floor is free from thick sediment for about 80 × 10^6 yr and is cross-cut with fractures and joints that permit seawater to circulate freely and react with basalt over long periods of time. Here, I emphasize some of the implications of these reactions.

The evidence for the reactions is both geochemical and geophysical. The chemistry of basalts sampled on the ocean floor varies progressively with distance from principal seafloor spreading ridges. Further, the seismic velocities of the uppermost rock layer of oceanic crust decrease progressively from the ridge, probably because of leaching of elements and hydration of the basalt layer by seawater. Similarly, the decrease in natural remnant magnetism of the seafloor with distance from the ridge can best be explained by a two layer model with the upper layer (0.5 km thick) exposed to seawater oxidation of the ferromagnetic minerals[2].

As a working hypothesis the rate of decrease of seismic velocity with distance from the oceanic ridges can be taken as a measure of the integrated rate of chemical weathering. Dividing this rate by the change of laboratory measured seismic velocity in basalt samples as a function of chemical changes, the rates of exchange for the principal elements can be computed. In general, the rates using the seismic velocity model are 1/3 to 1/2 the values observed by sampling basalts directly on the floor of the ocean (Fig. 1). This is expected if the rate of the basalt seawater reactions diminishes with depth.

As well as this weathering reaction, there is convincing isotopic evidence that greenschist facies metamorphic rocks have reacted with seawater, probably at greater depths than the weathering reaction[3,4]. Fig. 2 shows the chemistry of greenstones related to fresh basalts as a function of water content. There seem to be two distinct processes. The first, a primary stage which forms under the ridge crest, is characterized by the takeup of sodium and the loss of iron; a retrograde stage is dominated by a pickup of magnesium, iron and water.

Combining the weathering reaction with the primary and

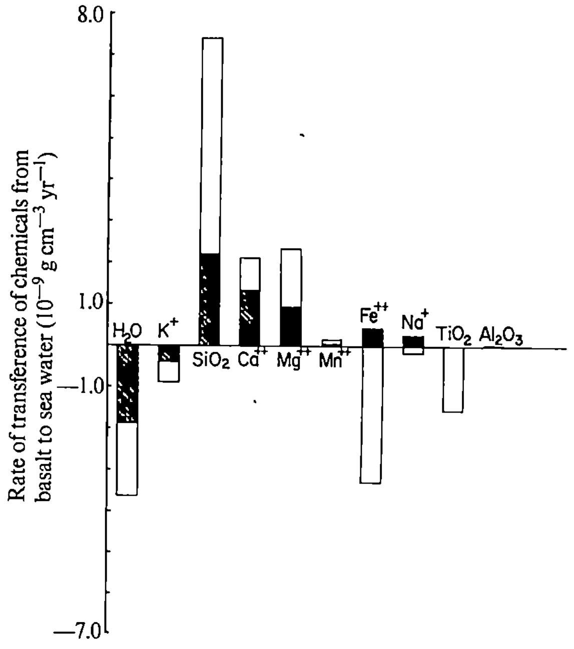

Fig. 1 ☐, Rate of transference calculated from surface samples; ■, rate from layer 2 seismic velocity. From ref. 1.

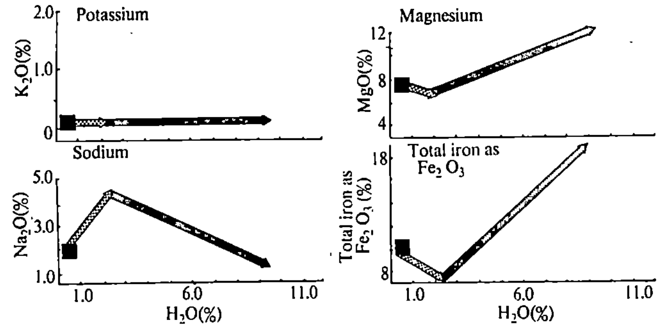

Fig. 2 Chemical changes produced by greenschist metamorphism of tholiietic ridge basalt[1]. ■, Fresh ridge basalt; dotted arrow, primary greenschist metamorphism (albite-actinolite dominant); cross-hatched arrow, retrograde greenschist metamorphism (chlorite dominant).

retrograde greenschist reaction, it is possible to write a general equation for the overall reaction between seawater and basalts of the seafloor for a reaction extending to a depth of 2.5 km. (Units are 10^{12} mol yr^{-1}.)

$$1.53\ K^+ + 3.75\ Mg^{2+} + 9.00\ Na^+ + 63.80\ H_2O + 194 \text{ fresh basalt} \rightarrow$$

$$187 \text{ altered basalt} + 16.69\ Ca^{2+} + 11.5\ H_2SiO_4 + 1.05\ Fe^{2+} + 0.14\ Mn$$

An important implication of the balance is that about 80% of the K^+, 60% of the Mg^{2+} and all the Na^{2+} from stream supply could be absorbed by the seafloor basalts while Ca^{2+}, SiO_2, Fe^{2+} and Mn^{2+} could be released to seawater in amounts comparable to stream supply. The Ca^{2+} and SiO_2 eventually find their way into the sediment pile by the action of diatoms, while the Fe^{2+} and Mn^{2+} are probably oxidized in metal-rich sediments and in manganese nodules.

I also emphasize that this reaction is most likely exothermic, and that the energy released at standard conditions can be estimated using the standard heat of formation of the cation species in aqueous solutions.

A minimum standard heat of reaction can be obtained by assuming that the heats of formation of solid products are equal to the solid reactants and a maximum by taking products to be on the average 100 kcalorie mol^{-1} less than the reactants. The minimum is 0.70×10^{16} kcalorie yr^{-1} and the maximum is 7.0×10^{16} kcalorie mol^{-1} yr^{-1}. It is possible to convert these values into standard heat flow units and compare them with a heat flow profile over the East Pacific Rise (Fig. 3). The observation that heat flow over the ocean floor is comparable with that measured over the more radiogenic continents is consistent with the suggestion that chemical reactions on the seafloor release energy comparable with the measured heat flow.

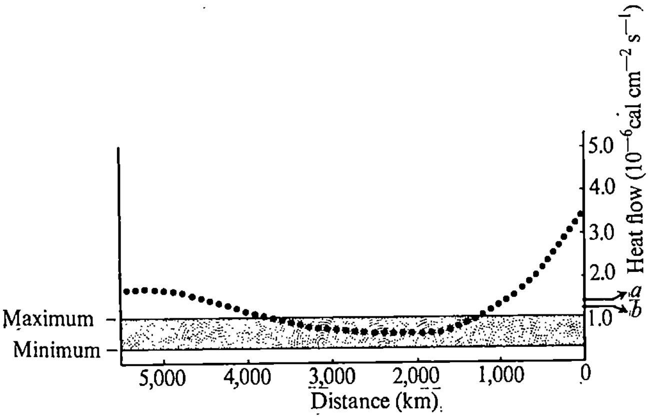

Fig. 3 ...., Heat flow profile over East Pacific rise[7]; shaded area, limiting band of chemical energy generation regime[1]. *a*, Pacific basin average; *b*, continental US average.

Finally I shall consider the possibility that changes in spreading could alter the chemical balance of the ocean and atmosphere. If the rate of spreading of the floors of the oceans increased, the volume of fresh basalt in contact with seawater would be increased and the potential for the chemical reactions would also become larger. The validity of the whole concept of chemical reactions on the seafloor could be tested by studying fluctuations in the chemistry of seawater as a function of time. Unfortunately, there are very few indicators of the chemical history of water that are not affected by secondary processes such as diagenesis. Two isotopic indicators that may be useful are the $^{87}Sr/^{86}Sr$ ratios measured in marine fossils, and $^{34}S/^{32}S$ ratios measured in evaporites. The measured values for these two isotope ratios through time are shown in Fig. 4. The isotope ratios do fluctuate with time, and the fluctuations for each element may be synchronous.

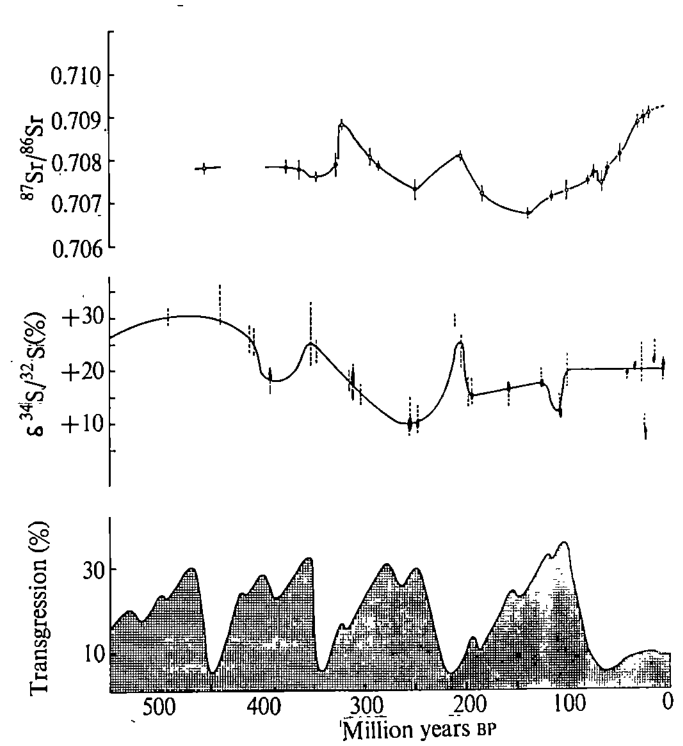

Fig. 4 Fluctuations of the strontium and sulphur compositions of seawater compared to epicontinental marine transgressions. Strontium isotope curve from ref. 8; sulphur isotope curve from Holser and Kaplan[9]; marine transgression curve from Damon[10].

Is it possible that these isotope fluctuations in seawater are caused by changes in seafloor spreading? There are no direct measurements of global changes in seafloor spreading; it has, however, been suggested[5] that times of high epicontinental sea transgression are times of high spreading rates; these are also plotted in Fig. 4.

Studies[5,6] show that fresh ridge basalt has a lower $^{87}Sr/^{86}Sr$ ratio than basalt that has been altered by seawater. The $^{87}Sr/^{86}Sr$ ratio of fresh ridge basalt is about 0.7026 and altered basalts have been measured with ratios as high as 0.7040. The times of fast spreading, as suggested by high marine transgression, roughly correspond to minima on the $^{87}Sr/^{86}Sr$ curve, suggesting the addition of non-radiogenic material from the basalt. Periods of slow spreading correspond, within the limitations of fossil dating, to maxima in the isotope curve because stream supply of radiogenic continental strontium overrides the supply from the basalt.

There is a similar approximate correspondence for the sulphur isotope curve, with minima on the curve matching times of faster spreading and maxima matching times of slower spreading. The explanation could be similar to the strontium case. The sulphur isotope ratios of basalt sulphide are lower than stream-supplied sulphate.

It also seems likely that changes in spreading rate would alter the residence times of K^+, Na^+ and Mg^{2+} in seawater and alter the supply of SiO_2, Ca^{2+}, Fe^{2+} and Mn^{2+} to seawater. In turn it is to be expected that the rate of deposition of these elements in seafloor sediments would vary. The anion species with which these elements combine during sedimentation, chiefly $CO_3{}^{2-}$ and O_2 would also vary. Therefore the CO_2 and O_2 content of the atmosphere could be dependent on conditions of seafloor spreading.

There is a need for more information on global changes in spreading rate and fluctuations in the chemistry of seawater; the data now available is, at least, consistent with the hypothesis of large scale reactions between seawater and basalt on the ocean floor.

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Compressive Stress between Two Mid-Ocean Ridges

ONE of the possible driving mechanisms of plate motions[1-3] is a push from mid-ocean ridges. This could arise from the injection of magma at the ridge crest[4], or to hydrostatic overpressure resulting from the elevation of the ridge above the ocean basins[5]. If the plates are pushed apart at ridges, an area between two mid-ocean ridges should be under compression. On May 9, 1971, an earthquake of magnitude 6.2 (m_b reported by NOAA) occurred within the Antarctic plate between the Chile Ridge and the Pacific–Antarctic Ridge, followed within 24 h by several aftershocks of magnitude 5.0 or greater (Fig. 1). An analysis of the focal mechanism of the principal shock shows that the plate is at least locally under horizontal compressive stress, with the maximum stress axis approximately perpendicular to the Pacific–Antarctic Ridge.

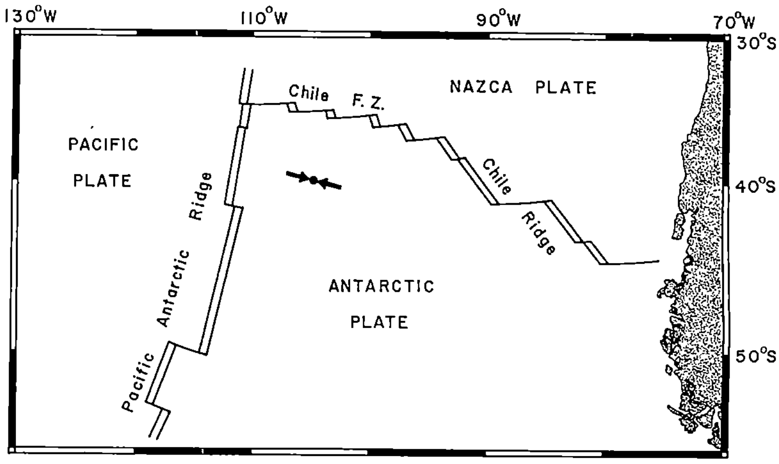
The S-wave polarizations and the P-wave compressional arrivals (Fig. 2) are consistent with predominantly thrust faulting. But the body wave data provide little control on the orientation of the fault and auxiliary planes. According to the P-wave data, the more steeply dipping plane, which for convenience I call the fault plane, could be assigned an orientation ranging from a strike of 175° east of north and a dip of 56° to a strike of 218° and a dip of 68°. There is almost no control on the other plane. Additional constraints on the source geometry were obtained from observations of Rayleigh wave amplitudes.

The amplitude of the Rayleigh waves (seismic surface waves) depends on the strike and dip of the fault plane and the direction of the slip on the fault. A plot of amplitude as a function for azimuth for a dip-slip source yields a two-lobed radiation pattern (Fig. 2). A strike-slip event would have a four-lobed pattern. By fitting theoretical radiation patterns to the observed azimuthal dependence of Rayleigh wave amplitude, the focal mechanism can be determined.

I measured the amplitudes by Fourier analysis of the long period vertical component of seismograph records from twenty-eight WWSSN stations, digitized at intervals of about 1.0 s. The observed amplitudes, corrected for attenuation, instrument response, and geometrical spreading on a spherical Earth, are plotted in Fig. 2. The overall pattern is two-lobed, reflecting the dip-slip nature of the event. Theoretical radiation patterns were generated according to formulas given by Saito[6] for the excitation of surface waves in a layered Earth, using an oceanic Earth model[7]. The least squares fit of the theoretical pattern to the observed amplitudes was computed for each source model. With the dip of the fault plane of approximately 60° determined by the body wave data, the slip and strike were allowed to vary until a number of acceptable models were found. A statistical F-test was performed comparing the fit of the best model with the fit of all other models. If, according to the F-test, there was a 90% probability that the best model was significantly better than some other model, then the other model was rejected. In this way, a range of acceptable models was outlined, with the boundaries defined at the 90% confidence limits. The region of possible models of the principal shock is shown in Fig. 3, with the 95% confidence limits also outlined.

Although the Rayleigh wave data do not yield any

Fig. 1 Location of the earthquakes of May 9, 1971. Epicentre of the principal shock is given by NOAA earthquake data report as 39.78° S, 104.84° W. The arrows indicate the direction of maximum horizontal compressive stress. Double lines represent actively spreading ridges, single lines are transform faults. The details of the plate boundaries in the Chile Fracture Zone are not well known. The strike of the Chile Ridge may be more north–south than is shown here (K. Klitgord, personal communication).



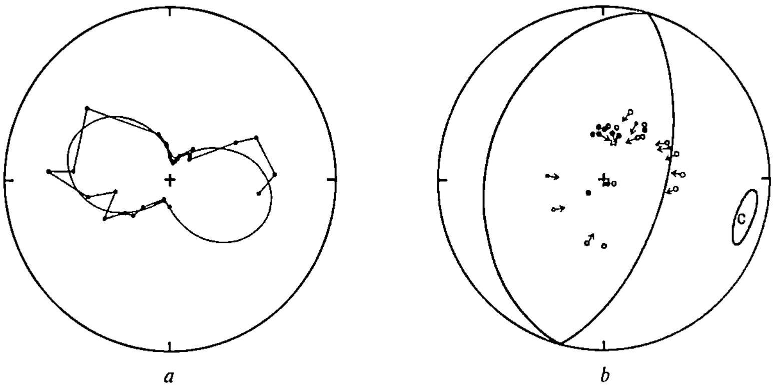


Fig. 2 Focal mechanism of the principal shock. In *a*, dots indicate observed amplitudes of 67 s Rayleigh waves as function of azimuth. The amplitude is proportional to the distance from the centre of the figure. The smooth continuous line shows the theoretical radiation pattern. *b* is a Schmidt net projection of the lower focal hemisphere showing the distribution of P-wave polarities and the direction of polarization of the S-waves. ●, Compressions; ○, dilatations. Smaller symbols represent poorer data. The small elliptical area surrounding the "C" indicates the uncertainty in the compressional axis. $Q=125$; strike=196°; dip=60°; slip=90°, depth=5.

additional limitation on the strike, the slip is found to be limited to 90 ± 25°. A slip of 90° represents pure thrusting, and a slip of 0° or 180° is pure strike-slip. But there is now the added constraint that, for any given strike,, the uncertainty in slip is only about ±10°. This constraint greatly reduces the uncertainty in the direction of the compression axis. The uncertainty in the compression axis is indicated by the ellipse in Fig. 2*b*. The strike of the compression axis is 105±11° east of north. The focal mechanism shown in Fig. 2 is the solution closest to the centre of the region of uncertainty.

In theory, the depth of the earthquake can be determined from the change in the radiation pattern with decreasing period of the Rayleigh wave. Because there is a large degree of scatter in the amplitudes for periods less than 20 s, the depth could not be precisely determined. Although the data seem to indicate that the shock was very shallow, less than 10 km, a detailed study of the effects of multiple path interference and attenuation would be required to determine the depth with certainty.

The event of May 9, 1971, is ideally located for a test of the hypothesis that plates are pushed apart at mid-ocean ridges. It is in perhaps the only area between two roughly parallel, actively spreading ridges, effectively isolating it from all other global tectonic processes. It is difficult to imagine the stress being generated by two convection cells upwelling under the ridge crests and flowing toward each other, without some surface expression at the point of juncture of the cells. The stress cannot be transmitted from another oceanic plate, because the ridge crests are tensile features decoupling the plates. The strike of the Pacific–Antarctic Ridge at 40° S is about 10° north of east[8], within 5° of being perpendicular to the axis of compressive stress. This observation and that of Mendiguren[9] of compressive stress within the Nazca plate can be explained most simply by failure at a weak point of a lithosphere under horizontal compressive stress caused by pushing from the mid-ocean ridges. A geographical traverse, which passed very close to the epicentre, found no obvious local tectonic feature which could create a local stress field[10]. Thus, the stress field found supports the hypothesis that pushing from the ridges is a primary driving mechanism of plate tectonics.

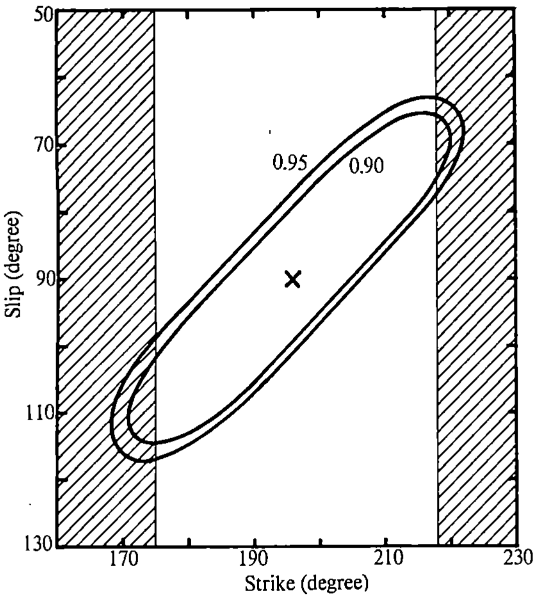


Fig. 3 Region of uncertainty in the fault plane solution. Based on analysis of 67 s period Rayleigh waves, only models with strike and slip within the inner elliptical region are considered acceptable. Models within the shaded regions are eliminated by the body wave data. The slip angle is measured counterclockwise from a horizontal line on the fault plane pointing in the direction of the strike. ×, Centre of region of uncertainty. Dip is 60°.

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Seasonal Variation of Underwater Noise in North-east Pacific

BEFORE marine seismic and other systems that rely on the detection of acoustic signals can be successfully designed, variation of ambient acoustic levels in the oceans throughout the year must be determined. Here I report measurements of ambient noise over a 1 yr period made recently at three sites in the North-east Pacific (Fig. 1). Remote instrument packages designed for a maximum water depth of 670 m, as previously used in relatively shallow Arctic waters[1], were placed near the summits of seamounts Bowie (summit depth about 40 m)[2], Union (~320 m)[2] and Cobb (~34 m)[3]. The package on

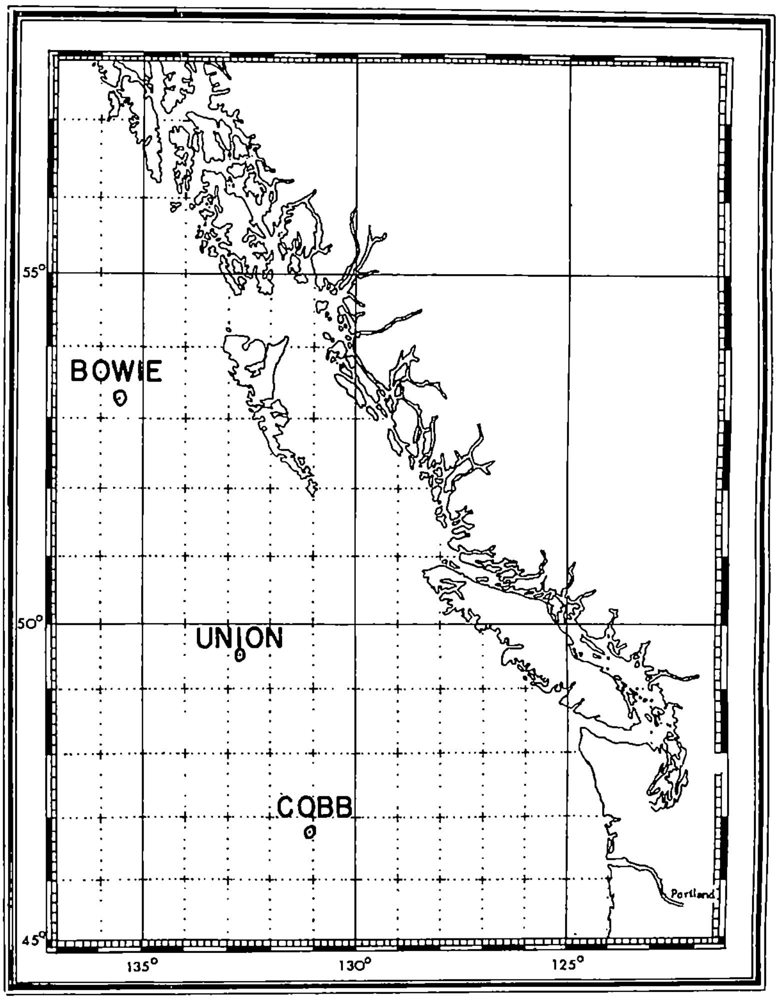

Fig. 1 Chart of the North-east Pacific showing the location of Seamounts Bowie, Union and Cobb.

Bowie was placed in a saddle—at the north-east end of the approximately 4 mile long ridge-like summit at a depth of 220 m. On Union the package was placed within 0.5 mile of a dome-like summit at a depth of 340 m and on Cobb the package was placed on a gently sloping terrace some 2 mile to the east of the summit, at a depth of 224 m. Acoustic measurements were limited to the frequency band 150 to 300 Hz. The data yield, although limited by equipment malfunction, was sufficient to reveal seasonal variations.

Four-minute observations of the noise sound pressure level were made every hour, averaged approximately using analogue circuitry over the observation period, and recorded as a single digital sample. Sampled values are presented in Fig. 2. An increase in noise level, which can be attributed at these acoustic frequencies almost entirely to storm activity and the associated high wind speeds, occurs in the winter months, although this trend is largely masked by the 3 to 6 dB standard deviation in the format of Fig. 2. The seasonal variation is best illustrated by plotting the monthly mean noise levels over the one-year observation period (Fig. 3). As well as the obvious seasonal trends, a uniform shift of about 8 dB is seen in the noise levels observed at Union Seamount relative to those observed at Cobb. This may well be attributable to the higher wind speeds likely to be encountered at the more northerly latitude of Union Seamount. But the limited data available from Bowie Seamount do not bear out this trend; the levels recorded at this site, the most northerly, fall between those measured at

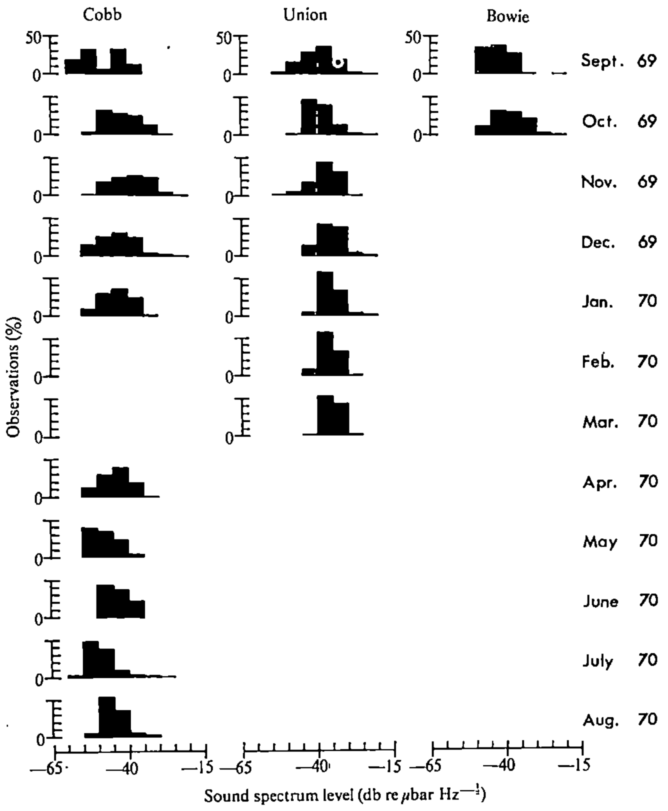

Fig. 2 Histograms of frequency of occurrence of ambient noise level for 5 dB increments of level for each month of the year and for each seamount. Equipment failure limited the useful data obtained at Union and Bowie Seamounts, and also for February and March, at Cobb.

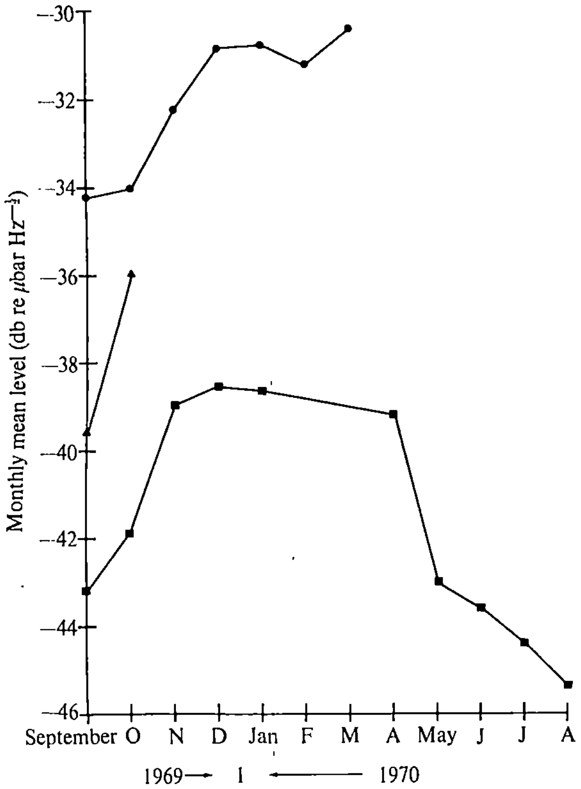

Fig. 3 Monthly mean ambient noise levels from September 1969 to August 1970. ●, Union; ▲, Bowie; ■, Cobb.

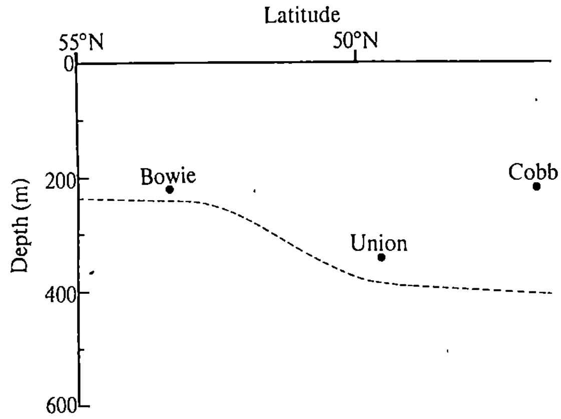

Fig. 4 Deep sound channel axis depth against latitude along an approximately straight line through the positions of seamounts Bowie, Union and Cobb. Instrument package latitudes and depth for the three seamounts are also plotted.

Union and Cobb Seamounts. The difference in prevailing noise levels may also be attributable to the different depths at which the sensors were located on the two seamounts and in particular to the fact that the deeper unit, located on Union, was situated close to the axis of the deep sound channel (Fig. 4). Under these circumstances an enhancement of ambient noise level at the depth of the sensor placed on Union Seamount is consistent with a hypothesis that much of the energy associated with underwater ambient noise is trapped in the sound channel.

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Point Defects and Extended Defects in Niobium Oxides

THE part played by true point defects—isolated vacancies and interstitial atoms—in defect solid solutions and in mixed valence non-stoichiometric phases is not clear. In many ionic or quasi-ionic structures there is some structural reorganization and at least incipient ordering of defect clusters. In some oxides—notably those of titanium, vanadium, molybdenum and tungsten, in which the covalent component of the bonding is probably strong—slight reduction, with a change in the ratio of oxygen to metal, is accommodated largely by crystallographic shear. A complete sheet of anion sites is eliminated from the structure, forming an extended defect and greatly diminishing the extent to which a deficit of oxygen must be compensated by anion vacancies or interstitial cations. Thus Hyde and Bursill[1] found that, in slightly reduced rutile, $TiO_{1.9986}$, the point defect concentration could hardly exceed 10^{-4}. In more extensively reduced rutile, changes in stoichiometry involve only changes in the spacing between crystallographic shear planes, without detectable concentrations of localized defects.

How far is the same true of the tightly defined crystal chemistry of the so-called "block structure" oxides, typified by niobium pentoxide? These constitute an extensive family of binary and ternary oxides, with structures essentially of the ReO_3 type, in which the anion/cation ratio is adjusted by crystallographic shear on (100) and (001) of the ReO_3 substructure. The whole is thereby divided into rectangular columns of ReO_3 structure, $(m\times n)$ coordination octahedra in cross-section, interfaced by crystallographic shear planes[2] (Fig. 1); these columns constitute subunits or building blocks that can be assembled into a wide variety of ordered or disordered structures with corresponding gradations of total composition. Much evidence is now available from lattice imaging electron microscopy to show this[3-7]. The dimensions $(m\times n)$ of the columns, and the way columns are linked at their corners, completely specify the stoichiometry of any perfect structure, but because it is topologically possible for different block structures, with different compositions, to intergrow coherently, very subtle changes of stoichiometry can be accommodated by the occasional intergrowth of lamellae of different structure. Fig. 2 shows one such extended defect: a lamella of $H\text{-}Nb_2O_5$ structure in a matrix of $MgNb_{24}O_{60}F_2$ (isostructural with $Nb_{25}O_{62}$). If no other, more localized, defects can be formed, it should be possible to account for deviations from the ideal composition of a given structure entirely in terms of coherent intergrowths.

It is, therefore, important that there is considerable evidence for variability of composition, in certain of these phases, that cannot be so explained. Gruehn[8] has shown that block structures with tetrahedrally coordinated cations in tunnels—and only those compounds—can have an analytically measurable, monophasic range, which does not necessarily include the ideal composition (Table 1). In a systematic lattice imaging study of the WO_3-Nb_2O_5 system, Allpress and Roth[9] could find no evidence of coherently intergrown "faults" in the $(4\times 3)_1$

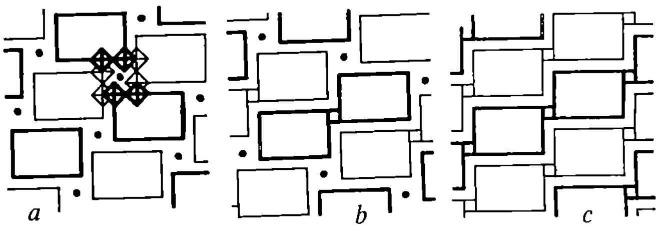

Fig. 1 *a*, Idealized structure of $WNb_{12}O_{33}$.[010] projection showing columns of octahedra, (4×3) in cross-section and the detailed structure of the tetrahedral site (●); *b*, structure of $Nb_{25}O_{62}$,[010] projection; *c*, structure of $Nb_{12}O_{29}$,[010] projection.

Table 1 Occupation of Tunnel Sites in Non-stoichiometric Block Structures

	Ratio of O to M Ideal	Observed limits	Composition for perfect column structure	Maximum occupancy number for tunnel sites
$WNb_{12}O_{33}$	2.5385	2.529–2.538	$M_{13\cdot05}O_{33}$	1.05
$Nb_{28}O_{70}$	2.5000	2.490 or 2.495–2.500	$M_{28\cdot12}O_{70}$ or $M_{28\cdot06}O_{70}$	1.12–1.06
$TiNb_{52}O_{132}$ $Nb_{53}O_{132}$	2.4906	2.483	$M_{53\cdot16}O_{132}$	1.08
$TiNb_{24}O_{62}$ $Nb_{25}O_{62}$	2.4800	2.472–2.478	$M_{25\cdot08}O_{62}$ to $M_{25\cdot02}O_{62}$	1.08 to 1.02
$Nb_{47}O_{116}$	2.4681	2.460	$M_{47\cdot15}O_{116}$	1.075
		2.464–2.467	$M_{47\cdot08}O_{116}$ to $M_{47\cdot02}O_{116}$	1.04 to 1.01
$Nb_{22}O_{54}$	2.4545	2.453	$M_{22\cdot014}O_{54}$	1.01
"$GeO_2.9Nb_2O_5$"	2.5000	2.474	$M_{10\cdot11}O_{25}$	1.11
"$P_2O_5.22Nb_2O_5$"	2.521	2.5000 (ref. 14)	$M_{23\cdot20}O_{58}$	1.06

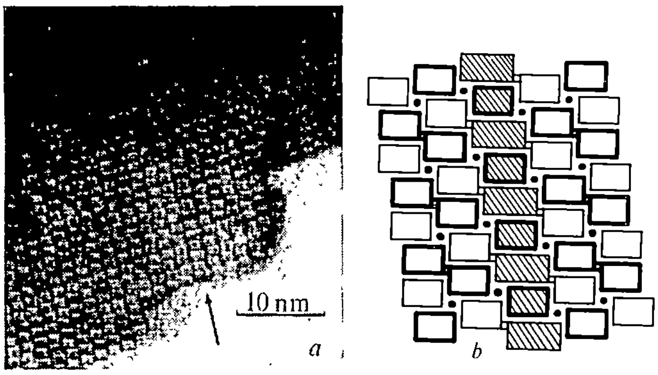

Fig. 2 *a*, Lattice image of $MgNb_{24}O_{60}F_2$ showing a lamella of H-Nb_2O_5 (arrowed); *b*, idealized structure of defect in $MgNb_{24}O_{60}F_2$.

block structure of $WNb_{12}O_{33}$ over the composition range $WO_3.6Nb_2O_5$ to $WO_3.8Nb_2O_5$ ($\equiv M_{13\cdot05}O_{33}$). In this laboratory we have found no intergrowths of $Nb_{25}O_{62}$ structure ($NbO_{2\cdot480}$) in slightly substoichiometric H-Nb_2O_5, down at least to the composition $NbO_{2\cdot495}$. All of these cases thus appear to represent genuine cation-excess structures.

An unambiguous example of an inherently defective block-structure is furnished by the phase "$GeO_2.9Nb_2O_5$" which Levin[10], from X-ray powder diffraction data, described as being isostructural with the $M_2O_5.9Nb_2O_5$ phases (M=P, As, V, Nb (ref. 11)). From the crystal structure of PNb_9O_{25} (ref. 12), these are $(3\times3)_1$ block structures (Fig. 3), with the atoms M occupying all the tetrahedral sites. As extreme possibilities, if the cation lattice in the germanium compound were complete, the formula would be $M_{10}O_{24\cdot74}$; if the oxygen lattice were filled, $M_{10\cdot11}O_{25}$. There must be either oxygen vacancies, or metal "interstitials," or both. We have, therefore, made a thorough study of this compound by lattice imaging electron microscopy, neutron diffraction and X-ray diffraction methods. Full results will be published elsewhere but some conclusions bearing on stoichiometric variability in block structure oxides, and on tunnel structures in general, may be reported now.

"$GeNb_{18}O_{47}$" is indeed statistically isostructural with PNb_9O_{25}, and its defect composition is not the consequence of coherent intergrowth with some other block structure. The (3×3) columns of ReO_3 structure are essentially perfect; oxygen sites and cation sites are fully occupied, and all the cations in the column are niobium. Hence the irrational composition is entirely due to an excess of cations within the tunnels. Examination of the structure shows that, instead of tetrahedral sites at $z=1/4$ and $3/4$, cations could occupy octahedral sites at $z=0$ or $1/2$; such sites occur in pairs, in two orientations. Our structure determinations prove that these octahedral tunnel sites are partially occupied. Cations in these positions, bridging the gap between columns at the same level (Fig. 4), must exclude the occupation of adjacent tetrahedral sites, and the limiting composition (determined from the occupation numbers) and structural considerations are compatible with (but do not prove) the hypothesis that tetrahedral cations and paired octahedral cations are ordered in regular sequence within each tunnel. There is no sign of superlattice ordering along the tunnel direction (the *b* axis of the structure); hence ordering within one tunnel must be quite uncorrelated with that in neighbouring tunnels.

Table 2 Cation Deficiencies on Tunnel Sites in Block Structures

Initial compound	Oxidation product		Composition if perfect block structure retained	Occupancy of tunnel sites
$Nb_{22}O_{54}$	OxIII	"$Nb_{22}O_{55}$"	$Nb_{21\cdot6}O_{54}$	0.6
$Nb_{47}O_{116}$	OxIV	"$Nb_{47}O_{117\cdot5}$"	$Nb_{46\cdot4}O_{116}$	0.7
$Nb_{25}O_{62}$	OxV	"$Nb_{25}O_{62\cdot5}$"	$Nb_{24\cdot8}O_{62}$	0.8
$Nb_{53}O_{132}$	OxVI	"$Nb_{53}O_{132\cdot5}$"	$Nb_{52\cdot8}O_{132}$	0.9

These findings make it worth examining the hypothesis that, in all these compounds, the ReO_3-like columns are perfect in structure, and that the non-stoichiometry recorded in Table 1 is due, in each case, to the "stuffing" of tunnels in the same way as in "$GeNb_{18}O_{47}$". That stoichiometric variability is confined to the tunnel structures would thereby be explained. Column 3 (Table 1) shows the compositions calculated on the basis of a perfect oxygen sublattice ; the consequential degree of maximum occupation of tunnel sites (column 4) is much the same as in the germanium compound. Having regard to some uncertainties in the real limiting compositions, this suggests that the limit is set by attainment of the same kind of ordering between octahedral and tetrahedral sites within each tunnel.

Tunnels can also be partially emptied of tetrahedral cations. Schäfer[13] showed that the reduced block structure oxides $Nb_{12}O_{29}$, $Nb_{22}O_{54}$, $Nb_{47}O_{116}$, $N_{25}O_{62}$ and $Nb_{53}O_{132}$ could be oxidized, each to its own distinctive modification of Nb_2O_5. Oxidation of $Nb_{12}O_{29}$, in which there are no tunnels of tetra-

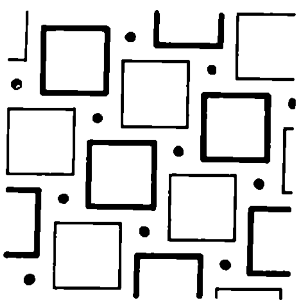

Fig. 3. Idealized structure of PNb_9O_{25}.

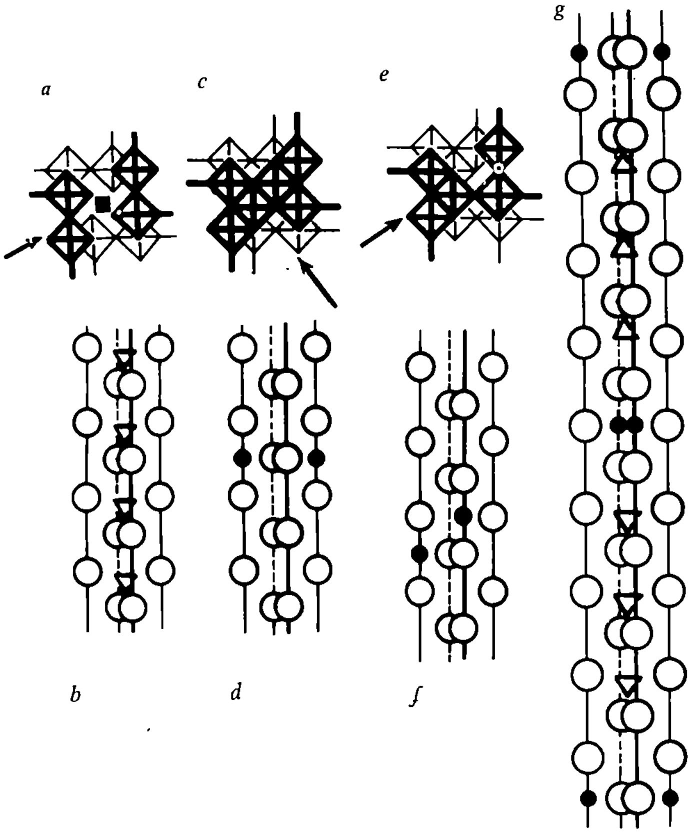

Fig. 4 *a*, Schematic projection of a tetrahedral site (■); *b*, projection of tetrahedral sites (▽) viewed in direction of arrow in (*a*). Oxygen atoms, (○); *c*, schematic projection of a pair of filled octahedral sites on one level; *d*, projection of filled octahedral sites (●) viewed in direction of arrow in (*c*); *e*, schematic projection of a pair of filled octahedral sites on different levels; *f*, projection of filled octahedral sites viewed in direction of arrow in (*e*); *g*, structure of "GeO$_2$.9Nb$_2$O$_5$".

hedral cations, follows a unique course[11]. We find, from electron diffraction and lattice imaging studies that the other materials, oxidized at low temperatures to avoid subsequent partial transformation to the thermodynamically stable H-Nb$_2$O$_5$, are crystallographically identical before and after oxidation. The block structures are unaltered. On the hypothesis that the ReO$_3$-like columns retain their integrity, Schäfer's forms of Nb$_2$O$_5$, OxIII to OxVI, must be represented as cation-deficient, as was proposed by Wadsley and Andersson[2] (Table 2). Whether filled and vacant cation sites order themselves within each tunnel is uncertain. No superlattice ordering has been detected after very long annealing periods; correlation between neighbouring tunnels is clearly very small, as in the case of tunnel stuffing.

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BIOLOGICAL SCIENCES

Immunogenic Properties of Solubilized Tumour Antigen from an RNA Virus-transformed Neoplasm

Numerous attempts have been made to solubilize tumour specific transplantation antigens (TSTA) of carcinogen-induced neoplasms and of virus-specified tumour antigens (VSTA) from the cell membranes of DNA and RNA virus-induced neoplasms[1-6]. Usually, yields of biologically active materials are low and the materials are labile. Soluble antigenic materials obtained from transplantable hepatomas[1,2] and purified by gel filtration were found to have limited biological activity. Although immunological specificity was preserved, the assays used were of relatively low precision. Tumour rejection, a major function of both TSTA and VSTA, was not observed.

Smith *et al.*[3] claimed the "first successful solubilization and fractionation of virus-induced TSTA". Inhibition of cytotoxicity *in vitro* was reported using 'Sephadex' G150 fractions of their antigen. But this activity was distributed over a wide range of low protein concentration and high molecular weight, so that no judgment of the molecular characteristic of this material can be made.

Crude extracts obtained by high speed centrifugation from tumours induced by carcinogens or by adenovirus 12 were reported to produce transplantation immunity[4-6]. It is improbable that "soluble" TSTAs were obtained by this procedure. Further purification of these materials has not been reported.

We have solubilized and partially purified histocompatibility antigens (H-2) of mice. After limited papain digestion followed by 'Sephadex' G150 column fractionation full biological activity was preserved and confined to a single peak[7-9]. These studies have provided, in our view, an excellent model and standard reference for the isolation and investigation of the immunological potentialities of soluble TSTA and VSTA.

Here we present evidence that a soluble tumour antigen isolated from cell membranes of RBL-5 leukaemic cells and partially purified can immunize animals so that specific tumour rejection occurs. The immunogenicity has been preserved through solubilization and purification.

RBL-5 leukaemic lymphocytes used as our source of tumour antigen (VSTA) have been passaged continuously in the ascitic form. This neoplasm arose in a C57Bl/6 mouse infected with Rauscher leukaemogenic virus (RLV). RBL-5 cells are immunogenic and provide immunity against other leukaemias of the FMR group (personal communication from

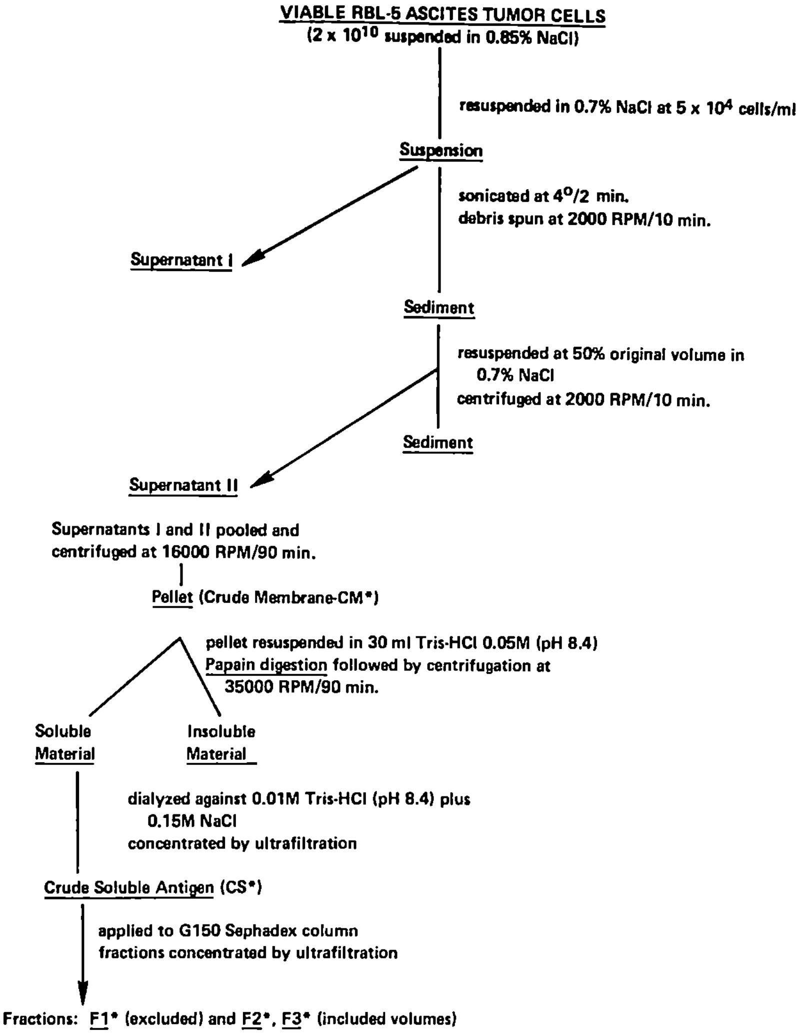
VIABLE RBL-5 ASCITES TUMOR CELLS
(2 x 10¹⁰ suspended in 0.85% NaCl)
resuspended in 0.7% NaCl at 5 x 10⁴ cells/ml
Suspension
sonicated at 4°/2 min.
debris spun at 2000 RPM/10 min.
Supernatant I
Sediment
resuspended at 50% original volume in 0.7% NaCl
centrifuged at 2000 RPM/10 min.
Sediment
Supernatant II
Supernatants I and II pooled and centrifuged at 16000 RPM/90 min.
Pellet (Crude Membrane-CM*)
pellet resuspended in 30 ml Tris-HCl 0.05M (pH 8.4)
Papain digestion followed by centrifugation at 35000 RPM/90 min.
Soluble Material
Insoluble Material
dialyzed against 0.01M Tris-HCl (pH 8.4) plus 0.15M NaCl
concentrated by ultrafiltration
Crude Soluble Antigen (CS*)
applied to G150 Sephadex column
fractions concentrated by ultrafiltration
Fractions: F1* (excluded) and F2*, F3* (included volumes)

Fig. 1 Flow chart of solubilization and chromatography on 'Sephadex' G150 of RBL-5 VSTA from crude membranes. (Details are given in the text.) Asterisks signify the materials assayed in this study.

J. L. McCoy). RBL-5 is also a good target cell; it is easily lysed *in vitro* by sensitized syngeneic lymphocytes[10], and, as determined in this laboratory, is lysed by specific cytotoxic antisera in the presence of complement; RBL-5 is not, however, very immunosensitive *in vivo* since immunized animals live only 1 or 2 d longer than controls. We have therefore used as challenge neoplasms in our *in vivo* assays LSTRA, an MLV (Moloney leukaemogenic virus)-induced leukaemia[11] and FBL-3, a Friend virus-induced leukaemia[12]. Both are known to contain virus-specified tumour rejection antigens (VSTA) of the FMR group[13]. CBF_1 mice (Balb/c × C57Bl/6), 12–16 weeks old, were used for tumour rejection studies since LSTRA is of Balb/c origin and FBL-3 is of C57Bl/6 origin; both leukaemias grew equally well in these F_1 hosts.

The methods used for extraction and further purification of soluble VSTA from RBL-5 membranes were similar to those used and tested in our studies of H-2 antigens[7–9] and are shown schematically in Fig. 1.

Ascites cells were collected routinely from 200 C57Bl/6 mice and red blood cells (RBC) were removed by Boyle's method[14]. Cells were washed twice with Tris-HCl, *p*H 7.4, 0.005 M; $MgSO_4$, 2×10^{-4} M; NaCl 0.85% (TBS), and their concentration was adjusted to 5×10^4 cells ml^{-1} in TBS solution containing 0.7% NaCl. A sonifier cell disruptor, Model W1850 (Heat Systems-Ultra Sonics, Inc., Plainview, New York) was used to disrupt the cells, which were then centrifuged at 600*g* for 10 min and the sediment resuspended in TBS-0.7% NaCl at 50% of the original volume. After 1 h the suspension was centrifuged as before. Supernatants were combined and centrifuged at 55,000*g* for 90 min. Pellets obtained were resuspended in 0.05 M Tris buffer, *p*H 8.4, at approximately 14 mg ml^{-1} and digested with papain at an enzyme: crude membrane ratio of 1 : 50 for 1 h at 37° C. The papain was activated with dithiothreitol at a final concentration of 0.01 M; the reaction was stopped with iodoacetamide at a final concentration of 0.022 M. The supernatant was separated from the insoluble sediment by centrifugation at 105,000*g* and was subsequently dialysed against 0.01 M Tris-HCl, *p*H 8.2, and 0.15 M NaCl before application to 'Sephadex' G150. The papain-solubilized material was concentrated by ultrafiltration using a UM-2 membrane (Amicon Corp., Lexington, Mass.). Protein determinations were carried out by dry weight and by the method of Lowry *et al.*[15]. An asterisk (*) denotes the materials in Fig. 1 that were used in our *in vivo* and *in vitro* studies.

Significant inhibition of the growth of both leukaemias, LSTRA (of Balb/c origin) and FBL-3 (of C57Bl/6 origin) in syngeneic CBF_1 mice was observed after immunization with crude membrane (CM), with the papain-solubilized CS preparation and with the F2 fraction obtained from 'Sephadex' G50 chromatography (Figs. 2–4). The F1 and F3 fractions used at the same concentrations as F2 were not immunogenic. These inhibitory effects were attained using tumour cell dosages for both leukaemias considerably above the tumour dose-50 (TD_{50}). The CM and CS preparations of RBL-5 cell membranes, administered in complete Freund's adjuvant, provided striking inhibition of the subcutaneous growths of LSTRA leukaemic cells as shown in Figs. 2*A* and *B*. The mean age to death was prolonged by 14.5±2.9 d in mice treated with our CM antigen preparation ($P<0.001$) and 11.8±3.48 d with the CS antigen preparation ($P<0.001$), comparing the group immunized with 100 µg with the Freund's adjuvant-treated controls. Inhibition of LSTRA growth by the CS preparation was also evident in the average diameter of the growths; for example, 10 d after LSTRA challenge the average diameter of the subcutaneous growths in adjuvant controls was 5.76 mm, of CBF$_1$ mice immunized with 100 μg CS, 3.26 mm, and of the group immunized with 50 μg CS, 3.28 mm.

When studying the inhibitory influence of 'Sephadex' G150 fractions on the growth of LSTRA leukaemic cells, advantage was taken of the delayed rate of growth of the localized subcutaneous growth as well as the reduced frequency of takes (Fig. 3*A*, *B* and *C*). Cumulative units of growth as used in studies summarized in Fig. 3 represent the product of the number of progressive takes per group by mean tumour diameter with time (see legend of Fig. 3). In Fig. 3*A*, crude soluble antigen (CS) was again found to provide inhibition. At day 8, after leukaemic cell challenge, for example, LSTRA had reached its maximum growth in the adjuvant-treated controls whereas CS antigen-treated mice had no tumours. Resistance to the growth of LSTRA in mice pretreated with the 'Sephadex' G150 fraction, F2, is summarized in Fig. 3*B* and *C*. Those BDF$_1$ mice inoculated with 4.4×10^3 LSTRA cells and pretreated with the F2 fraction (Fig. 3*C*) showed a reduced frequency of takes and slower tumour growth when compared with controls and those mice pretreated with the F1 and F3 'Sephadex' fractions. The difference in mean units of growth observed 20 d after LSTRA challenge, between the F2 antigen recipients and the other three groups combined was 0.85 ± 0.40 ($P<0.04$); also two out of seven mice did not grow LSTRA whereas LSTRA grew progressively and killed nineteen out of twenty-one mice of the combined groups.

FBL-3 lymphocytic leukaemia induced by Friend virus and carried as an ascitic neoplasm in C57Bl/6 mice is known to contain type-C virus particles and possesses membrane tumour antigens (cellular or virion) which cross-react with other leukaemias induced by the FMR group; this leukaemia disseminates widely after intraperitoneal challenge with inoculations as low as 1×10^4 cells. CBF$_1$ mice pretreated with both the CM and F2 fraction preparations but not with F1 and F3 showed a striking resistance to FBL-3 challenge at cell concentrations of approximately the TD$_{80-90}$ as reflected in frequency of leukaemic deaths. The difference of the mean frequencies of death in Fig. 4*A* was $70\pm23\%$ ($P<0.002$) and in Fig. 4*B*, $62.5\pm16.2\%$ ($P<0.001$) for the three groups combined, adjuvant control plus F1 and F3 fraction recipients versus the F2 fraction recipients. This activity of the F2 fractions observed in these *in vivo* studies correlated with the activity of F2 in inhibiting cytotoxicity of RBL-5 cells *in vitro*, as discussed below.

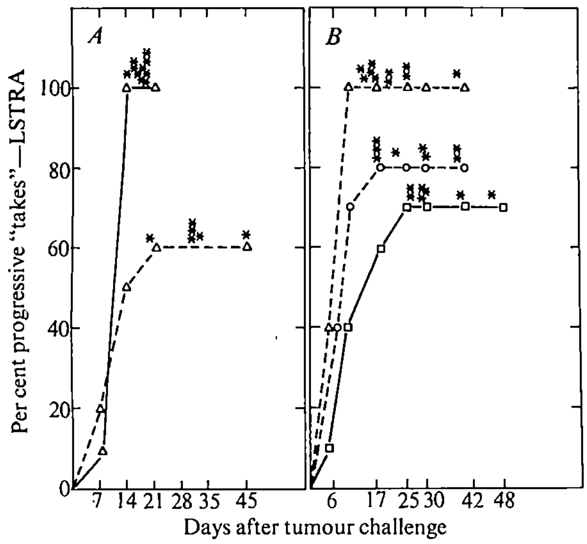

Fig. 2 Inhibition of growth of LSTRA leukaemia in 12-week-old CBF$_1$ female recipients. Data in *A* represent combined groups receiving 2.9×10^3 and 5.8×10^3 ascitic cells inoculated subcutaneously; data in *B* represent combined groups inoculated subcutaneously with 4.4×10^3 and 2.2×10^3 cells respectively. The TD$_{50}$ for LSTRA leukaemic cells is $<1.0\times10^3$ cells. *A*: △---△, mice inoculated intraperitoneally with 400 μg CM antigen in complete Freund's adjuvant from RBL-5 leukaemic cells; two antigen inoculations were given 7 d apart and challenge with LSTRA was made 14 d after the last immunization. Control mice (△—△) received Freund's adjuvant. Asterisks above curves represent days to death with neoplasm. The mean time to death in CM-immunized mice was 32.0 ± 2.9 d and in controls, 17.5 ± 0.51 d. The period of observation was 90 d. *B*: ○---○, CBF$_1$ female mice immunized with 50 μg of crude soluble (CS) antigen in Freund's adjuvant ×3 at weekly intervals; □—□, immunization with 100 μg CS×3; △---△, control mice inoculated with Freund's adjuvant only. The mean time to death of the control group was 20.5 ± 2.2 d, of the 50 μg group, 26.0 d and of the 100 μg group, 32.3 ± 2.7 d.

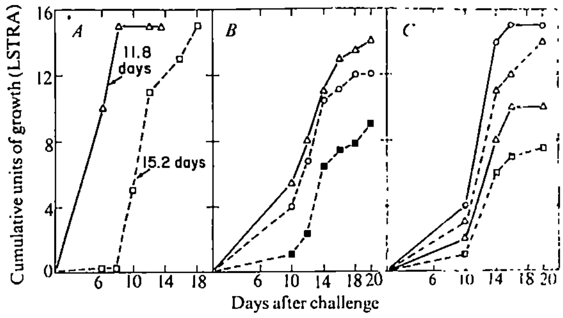

Fig. 3 Inhibition of the growth of LSTRA in CBF$_1$ female mice 12 weeks old immunized with antigen and with 'Sephadex' G150 fractions: F1, F2 and F3. Inhibition of growth was reflected in the rate of growth of the localized tumour as well as in the frequency of progressively growing takes; thus, cumulative units of growth represent the product of the number of takes per group by the mean average diameter of the subcutaneous growth. Units of mean diameter, used for simplification, were: 0.5 (5×5×5 mm), 1 (10×10×15 mm), 2 (15×15×25 mm) and 3 (25×25×40 mm or greater). Maximum growth obtained in five mice per group was therefore 15 cumulative units. *A*: □---□, five mice inoculated with 50 μg CS antigen in complete Freund's adjuvant weekly ×3; △—△, five control mice received complete Freund's adjuvant only. Challenge, subcutaneously with 2.3×10^3 LSTRA cells was made 2 weeks after the last immunization. The mean time to death of immunized mice was 15.2 d and of control mice, 11.8 d. *B*: ■---■, CBF$_1$ mice immunized with the 'Sephadex' G150, F2 fraction, 100 μg in complete Freund's adjuvant inoculated ×2, at 1 week intervals; ○---○, mice inoculated with the F3 fraction at 100 μg ×2; △—△, controls received complete Freund's adjuvant. There were ten mice in each group, half of which were inoculated subcutaneously 2 weeks after the last treatment with 1.2×10^4 LSTRA leukaemic cells and half of which received 6×10^3 cells. Thus, cumulative units of growth represent the means of these two subgroups of five each within each group. *C*: ■---■, CBF$_1$ mice inoculated with the 'Sephadex' G150 F2 fraction at a concentration of 100 μg in Freund's complete adjuvant ×2; ○—○, F3 fraction at same concentration and schedule; △---△, F1 fraction; △—△, Freund's adjuvant-inoculated controls. Challenge of 4.4×10^3 LSTRA cells was made 2 weeks after the last treatment. There were seven mice in each group, thus, maximum cumulative units of growth here is 21.

To test for the specificity of the immunogenic response to RBL-5 membrane antigen, C57Bl mice were pretreated with the CM preparation at the concentration and schedule in which resistance to leukaemic cell growth of LSTRA and FBL-3 was achieved. Challenge with syngeneic tumour No. 89 known to contain a polyoma virus-specific surface antigen[16] did not reveal inhibition of the growth of this neoplasm (Table 1).

Antisera obtained from CBF$_1$ mice that received inoculations weekly for 3 weeks with 400 μg of the CM preparation were cytotoxic for ^{51}Cr-labelled RBL-5 target cells in the presence of rabbit complement. This cytotoxicity titre increased from 1/32 to 1/128 (80% lysis) with continued immunizations over a further 3 weeks. These antisera did not neutralize RLV. Neither infectious RLV, as assayed in the indirect XC test[17] nor type-C particles from electron microscopic study were observed in our CM preparation.

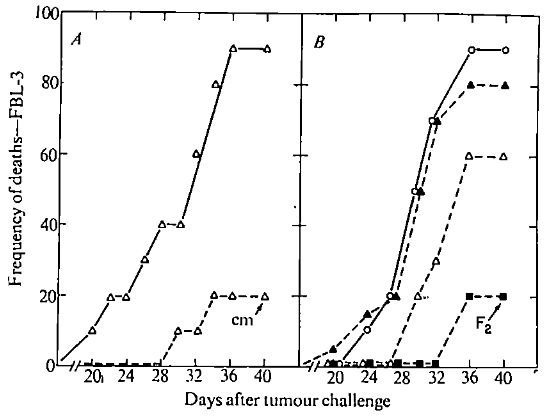

Fig. 4 Inhibition of growth of Friend virus-induced lymphocytic neoplasm, FBL-3, CBF$_1$ male mice 12 weeks old. All challenge inoculations were made intraperitoneally from the ascites-maintained neoplasm 2 weeks after the last treatment subcutaneously with the RBL-5 antigen preparations. *A*: △---△, 400 µg CM antigen in complete Freund's adjuvant inoculated subcutaneously ×2; △—△, Freund's adjuvant-treated controls. Groups receiving 1×10^5 and 5×10^5 FBL-3 cells are combined. There were ten mice in each group. *B*: ■---■, mice treated with the F2 fraction, 100 µg ×2 at weekly interval; ○—○ and △---△, mice treated with the F3 and F1 fractions respectively, both at same concentration and schedule as F2; ▲---▲, Freund's adjuvant-treated controls. There were ten mice in each group and twenty in the control group. Results for groups receiving 5×10^5 and 1×10^6 FBL-3 cells are combined. The TD$_{50}$ of FBL-3 was approximately 1×10^4 leukaemic cells. At 75 d after FBL-3 leukaemic cell challenge five out of ten (50%) of the F2 fraction-treated mice were alive and free of tumours, compared with only seven out of forty (17.5%) of the other combined groups.

In *in vitro* complement-dependent cytotoxic assays using RBL-5 target cells labelled with ^{51}Cr, inhibition of lysis was observed repeatedly using the CM preparation, the papain-solubilized CS and the pooled F2 fraction of antigen. A high-titred anti-MSV(MLV) antiserum and the antiserum produced in syngeneic mice after repeated CM immunizations, as discussed above, were used interchangeably. These antisera were not cytotoxic for normal lymph node cells of C57Bl/6 origin nor for SV-AL/N fibroblasts known to contain SV40 VSTA[18]. The pooled F1 and the pooled F3 fractions at the same concentrations (4 to 10 mg protein ml^{-1}) and in identical conditions did not inhibit cytotoxicity.

We tried to calculate the overall yield of activity of this VSTA using inhibition of ^{51}Cr release (see ref. 18). If inhibition titres obtained with the CM antigen preparations are used (titres ranging from 1/64 to 1/128 at 50% inhibition of lysis), the F2 fraction contained 4 to 6% of the CM total activity. Activity was found, as assayed by inhibition of cytotoxicity, in the sediment and this is an indication that our papain digestion has not destroyed the activity as measured *in vitro*; attempts are now underway to solubilize this remaining activity by methods other than papain digestion.

In the pooled F2 fraction as well as in the CM preparation, H-2 specificities 2 and 28 were also detected using monospecific alloantisera. The activity we observed in our cytotoxicity assays coincides with the pooled F2 fraction of H-2 specificities (see Fig. 5).

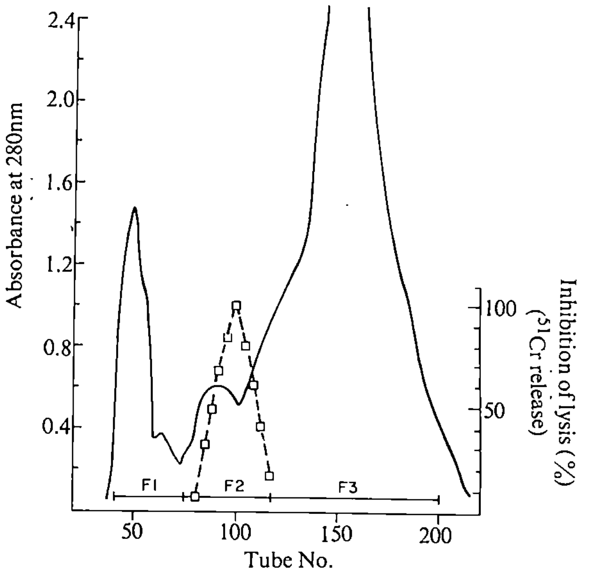

Fig. 5 Fractionation of papain-solubilized VSTA from 2×10^{10} RBL-5 ascitic cells on 'Sephadex' G150 column (2.3×150 cm) which was equilibrated with and eluted with 0.01 M Tris-HCl buffer, *p*H 8.2, and 0.15 M NaCl. ——, Absorbance at 280 nm; □---□, individual inhibitory activities of H-2.28 alloantigenic specificity. The peak F2 when pooled at 4 mg ml^{-1} concentration was the only fraction containing inhibitory activity as assayed in the *in vitro* cytotoxicity test; peak F1 and F3 fractions were negative.

An obvious source of tumour antigens of the transplantation type in neoplasms induced by RNA viruses of the leukaemia-sarcoma complexes is infective virus or a structural part of the virus, since productive, non-lytic infection continues indefinitely in these induced neoplasms in contrast to neoplasms induced by DNA oncogenic viruses. Thus, expression of VSTA could be an expression of replicating, intact, infective virus incorporated in the cell membrane. Previous work from this laboratory[19] and others[20,21] using "non-releaser" neoplasms shows that VSTAs, at least of some RNA virus-induced tumours are of non-virion origin, but are nevertheless specified by the virus inducing the neoplasm.

Table 1 Test for the Specificity of RBL-5 Tumour Antigen C57Bl Mice Immunized with RBL-5 Crude Membrane Antigen. Challenged with Polyoma-specific Neoplasm No. 89

Group	Treatment	No. polyoma tumour cells inoculated	No. with tumour / No. in group	Mean tumour diameter (mm at days): 14	28	35	50
1	Crude membrane	5×10^4	5/5	7.2	8.3	12.3	16.2
	400 µg	1×10^4	4/5	6.7	6.2	7.9	11.5
2	Freund's adj. only	5×10^4	5/5	8.3	8.1	13.3	18.3
	(Controls)	1×10^4	4/5	7.4	6.6	6.8	11.4

Tumour No. 89 containing polyoma virus-specific surface antigen[16] was inoculated subcutaneously into syngeneic C57Bl/Ka female mice 14 d after last treatment with the crude membrane (CM) antigen preparation. The concentration of 1×10^4 tumour cells was determined in previous experiments to be approximately the TD$_{60}$. The mean tumour diameter is the arithmetic mean of length+width+depth. The time to death with neoplastic growths for the combined (5×10^4 and 1×10^4 groups) was 54.0 d for group 1 and 53.4 d for group 2. Tumour No. 89 failed to grow in 13 C57Bl/Ka mice immunized with polyoma virus and inoculated with 2×10^4 dissociated No. 89 cells while nine out of fifteen (60%) untreated controls died of progressively growing tumours 56 d after grafting.

The tumour antigens isolated from RBL-5 discussed here, and responsible for the specific immunological reactions described seem also to be of nonvirion origin. The CM material did not contain infective leukaemogenic virus as determined in the indirect XC assay, CM-immunized mice did not produce antiviral antibodies, as assayed in Rauscher-virus neutralization studies nor were any type-C particles observed in our CM preparations.

We conclude from these findings that a molecular entity with the characteristics of a tumour antigen of the transplantation type has been solubilized from a crude membrane preparation. Immunogenic properties of the VSTA have been preserved and this entity does not seem to be a virion antigen or a structural part of RLV. From the characteristic elution on 'Sephadex', this entity seems to be of relatively high molecular weight (about 50,000–60,000); it elutes in the same area in which both H-2 and non-H-2 activities have been shown to elute after solubilization with papain[7].

As it is very unlikely that we are measuring an H-2 or non-H-2 activity (in our syngeneic system), the VSTA solubilized in these studies must represent a new surface component (antigenic determinant) that can be isolated and further analysed. This analysis we hope will shed some light on the mechanisms involved in the interaction between viral genes and cell regulating mechanisms.

We thank Donald Foor, Sam Morris and Thomas Fischetti for technical assistance. Dr Kenneth Chang performed the assays for infective RLV and for neutralizing antibodies to RLV; Dr Nelson Wivel performed the electron microscopic study for type-C particles. We thank Dr D. Lavrin for anti-MSV(MLV) antisera.

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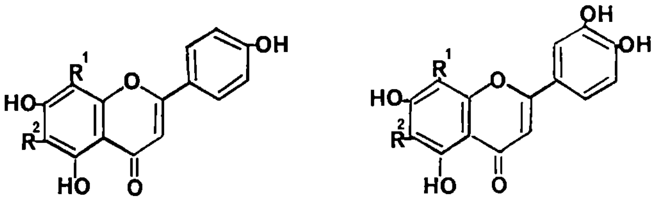
Polyploidy and Flavonoid Synthesis in *Briza media* L.

POLYPLOIDY may increase the flexibility of a species and consequently its ecological range by extending physiological tolerance[1,2]. In *Lycopersicon esculentum* polyploidy is reported to raise ascorbic acid levels in the fruit[3] and in *Valeriana officinalis* the polyploids have been shown to be better adapted to certain soil types than the diploids[4]. Little work, however, has been carried out on chemical differences between chromosome races, so it was of some interest, when we found that diploid and tetraploid plants of *Briza media*, which have different geographical distributions also have distinct leaf flavonoid patterns. Diploid plants accumulate the *C*-glycosylflavones vitexin and isovitexin, tetraploid plants orientin and iso-orientin (Fig. 1).



I II

Fig. 1 Structures of flavonoids found in polyploids of *B. media*. I, vitexin, R^1 = glucose, R^2 = H; isovitexin, R^1 = H, R^2 = glucose. II, Orientin, R^1 = glucose, R^2 = H; iso-orientin, R^1 = H, R^2 = glucose.

Here we report on the leaf flavonoid patterns of artificially produced polyploids, differences in enzyme activity with ploidy level, the discovery of a new isomerase enzyme converting isovitexin to vitexin and possible effects of polyploidy on the natural distribution of *Briza media*.

The plants used were collected in the wild or grown from spontaneous seed. Artificial tetraploids were produced by colchicine treatment of seedlings and chromosome numbers obtained by root tip squashes[5]. Flavonoid identification has been described previously[6]. Hydroxylase activity was assayed by a modified version of the method of Vaughan and Butt[7] using acetone powder enzyme extracts instead of a *brie* to remove interfering substrates present in the leaf. The powder was further purified by precipitation at 50% (fraction 1) and 100% (fraction 2) saturation with ammonium sulphate in 0.05 M citrate–phosphate buffer *p*H 5.4 Horizontal block electrophoresis of leaf and seed proteins was carried out on a 7.5% acrylamide gel, after the method of Lund[8] as described by Crowden *et al.*[9].

Leaf flavonoids have been examined in several artificially produced polyploids of *B. media;* triploids, formed by crossing naturally occurring tetraploids with diploids, artificial tetraploids and octoploids were all found to synthesize orientin isomers and indeed to have an identical chromatographic pattern with the naturally occurring tetraploid. Thus it seems that the addition of a single genome is sufficient to cause a change in flavonoid synthesis in *B. media* and the addition of more genomes has no further effect.

Vitexin and orientin are *C*-glycosylflavones differing in the number of hydroxyl groups on the B-ring (Fig. 1) but it is not known whether they are formed by consecutive steps in the same biosynthetic pathway[10]. There is evidence, however, that flavones (with no *C*-sugar) containing a 4′-monohydroxy B-ring can be oxidized to flavones with a 3′,4′-dihydroxy B-ring in the families Lemnaceae[11] and Umbelliferae[12]. *C*-Glycosylation appears to occur at a much earlier stage in biosynthesis than both *O*-glycosylation and *O*-methylation[11].

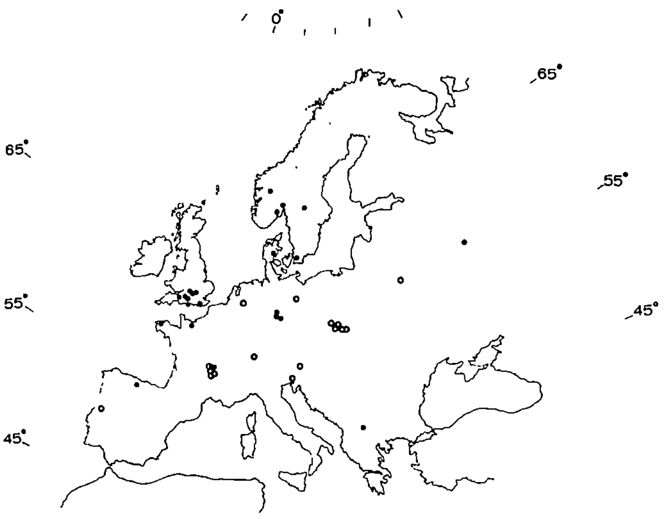


Fig. 2 Distribution of diploid (●) and tetraploid (○) races of *Briza media* in Europe.

All attempts at isolating an enzyme from *B. media* for hydroxylating vitexin to orientin failed but during the course of these experiments a totally different enzyme activity was discovered. Thus isovitexin, when incubated at 37° C with the partially purified enzyme fractions at *p*H 5.4, was completely converted to vitexin in 1 h. The conversion was monitored between 0 and 6 h by paper chromatography using standard solvents[10]. Neither the reverse reaction nor the conversion of iso-orientin to orientin could be shown. Boiling for 20 min stopped the reaction completely in both diploid fractions and tetraploid fraction 2. Fraction 1 (tetraploid) remained active but was inactivated by 2 h boiling. Buffer controls (boiled and unboiled) showed no conversion of isovitexin to vitexin. The optimum *p*H for the reaction was around 6.8, at which conversion was almost instantaneous. At *p*H 2.6 the reaction was very slow and at *p*H's 4.0, 5.4 and 8.8 it was complete in 15 min (fraction 1) and 1 h (fraction 2), at *p*H 2.6–6.8 citrate-phosphate buffer and borate buffer *p*H 8.8. Neither vitexin nor the buffer controls showed conversion at any *p*H. It thus seems that the leaf of *B. media* contains an isomerase enzyme or enzymes converting isovitexin to vitexin and differing with ploidy level in heat stability. This is the first report of such an enzyme.

The function of the new enzyme in the plant is not yet clear, but it does not seem to have an important synthetic role, as the ratio of isovitexin to vitexin in leaf tissue is very much in favour of the 6-isomer, the ratio being approximately 4:1. Furthermore Wallace and Mabry[13] have shown by tracer feeding experiments in *Lemna minor* that vitexin is converted to isovitexin *in vivo* but were unable to demonstrate the reverse reaction. The isomerase, therefore, may be more important in the metabolism and turnover of flavone *C*-glycosides and this is being further explored.

The proteins of diploid, triploid and tetraploid seeds were compared using horizontal block electrophoresis. Esterase and peroxidase activity was determined according to the method of Crowden *et al.*[9]. Esterase activity was found to be about four times greater in triploid and tetraploid leaves than diploid but peroxidase activity was equally strong in all three. Seed extracts were weak in esterase activity but the diploid gave two distinct bands, the tetraploid only one. The seed peroxidase reaction did not vary with ploidy but it was interesting that both cationic and anionic bands were observed.

It appears, therefore, that in *B. media* polyploidy affects some physiological processes such as flavonoid synthesis, esterase activity and the heat stability of the new *C*-glycosylflavone isomerase but does not affect peroxidase activity or the actual isomerase reaction. These physiological changes may be in some way enabling tetraploid races to extend the range of the species by exploiting different climatic and edaphic zones. The geographic separations of the races (Fig. 2) would appear to support this idea. The tetraploids are most common in "central" Europe and have not been found in Britain or Scandinavia where all populations studied were diploid. The two races have only been found growing sympatically in the Massif Central of France[14]—a botanically and geologically unusual area.

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Ethanol inhibits Intestinal Calcium Transport in Rats

Although it is known that intestinal calcium malabsorption may occur in chronic alcoholic patients indirectly as a result of steatorrhoea of multiple causes[1–7], producing inadequate absorption of fat-soluble vitamin D, little attention has been paid to possible direct effect on small intestinal transport. To test that possibility, I starved male albino rats 8 h before and 18 h after the acute administration of ethanol (7.5 g kg^{-1} body weight) or isocaloric glucose (13.7 g kg^{-1}; they were, however, permitted free access to water. Chronic ethanol ingestion was achieved by giving 20% ethanol in water as a sole source of fluid and standard laboratory chow was fed *ad libitum* for 12 d. Comparisons were made with *ad libitum* and pair-fed controls. Calcium transport by duodenum and ileum was measured using an *in vitro* everted gut sac technique[8].

The results of experiments measuring serosal/mucosal ^{45}Ca concentration ratios in animals given the single dose of ethanol (Fig. 1) indicate no significant difference in transport ratios ($P>0.05$). In animals ingesting ethanol chronically there was no significant difference between the weights of pair-fed animals (124 ± 3 g) and ethanol-fed animals (124 ± 4 g) although both were statistically depressed compared with animals fed *ad libitum* (175 ± 5 g) ($P<0.01$). Chronic administration of ethanol interfered with the ability of duodenum to transport calcium ($P<0.01$) compared to *ad libitum* or pair-fed controls (Fig. 1). The difference between the two control groups is not statistically different. I found no differences in ileal concentration ratios ($P>0.05$). Examination of liver and intestines by light microscopy revealed normal histology in all groups and determinations of serum bilirubin, alkaline phosphatase and aspartate aminotransferase (GOT), which were performed to monitor hepatic function, were normal in the three groups.

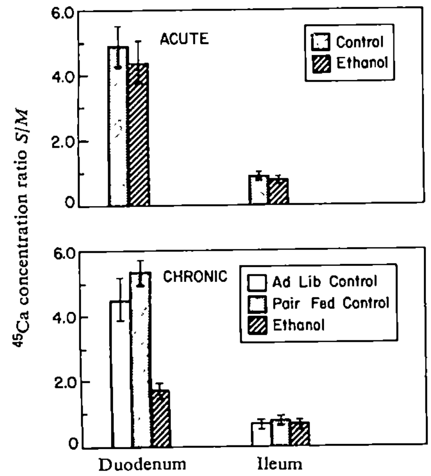

Fig. 1 Effect of ethanol *in vivo* on calcium transport by rat small intestine. Sacs 5 cm long, filled with 0.5 ml of a medium consisting of 0.125 M NaCl, 0.01 M fructose, 0.00025 M $CaCl_2$, 0.030 M Tris-Cl, *p*H 7.4, and sufficient $^{45}CaCl_2$ to provide approximately 25,000 c.p.m. ml^{-1}, were incubated at 37° C, with continuous bubbling of 100% oxygen, for 90 min, in 25 ml Erlenmeyer flasks containing 10 ml of identical medium. Radioactivity was determined by liquid scintillation spectrometry. Data are expressed as a final concentration ratio of the tracer inside the sac (serosal medium) over that outside the sac (mucosal medium), *S/M*. Eight or nine animals were used in each group. Values represent mean standard error.

It seems that the chronic ingestion of ethanol directly inhibits active transport of calcium by the rat duodenum, that segment which has been shown to most avidly absorb calcium[9,10]. Comparison to pair-fed malnourished controls indicates the effect is independent of partial starvation. No evidence of liver dysfunction or cholestasis was present as measured by histological appearance and liver function tests. Pancreatic dysfunction cannot be implicated in these studies because chronic pancreatitis appears only after the twentieth month of exposure[11]. A direct toxic effect might be responsible for this direct interference, but the normal histology in these studies, as well as the normal appearance of duodenum and jejunum observed previously in humans[12-15], indicate a lack of structural changes as evidenced by light microscopy. In rats ingesting ethanol for 9 to 12 months, however, ultrastructural changes in ileum have been recognized, in spite of normal light microscopic appearance[16]. The mechanisms underlying this inhibition of calcium transport and the clinical implications remain to be determined.

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The Regulation of Intestinal Calcium Transport by Vitamin D

THERE is much evidence that metabolites of vitamin D are fundamentally involved in calcium absorption by the intestine. Cholecalciferol (vitamin D_3) is hydroxylated at C_{25} in the liver[1,2] and then at C_1 in the kidney[3-5]. 1,25-dihydroxycholecalciferol is thought to be the active form, a hormone which promotes Ca transport by the intestine[5-8]. Ca translocation across the mucosal epithelial cell involves entry across the brush border and exit across the basal-lateral membrane. The site and manner of action of vitamin D metabolites has not been unequivocally established, but has been suggested to be at the basal-lateral membrane[9], the brush border[10,11], or both[12,13]. Kinetic studies in the normal rat led to the conclusion that entry across the brush border was rate-limiting for Ca translocation across the epithelium[14]. This would itself suggest that 1,25-dihydroxycholecalciferol regulated Ca absorption by acting at the brush border.

Male albino rats were fed from weaning on a vitamin D-deficient diet. This consisted of per kg, 528 g whole wheat flour, 192 g wheat germ, 48 g yeast extract, 48 g casein, 144 g sucrose, 18 g olive oil, 12 g $CaCO_3$, 9.6 g NaCl, 10 mg ascorbic acid, 200 mg ferrous lactate, 200 mg $MgSO_4$, 5 mg $MnSO_4$, 5 mg $CuSO_4$, 1 mg KI, 0.3 mg $CoSO_4$ and 3,000 I.U. vitamin A. The diet contained 0.52% Ca and 0.50% P.

Parsons has shown that everted duodenal sacs from rats fed this diet from weaning to six to eight weeks old showed no active transport of ^{45}Ca with mean serocal/mucosal concentration ratio = 1.2. Active transport occurred when the rats were dosed with 400 I.U. vitamin D: the mean ratio increased to 2.7 after 8 h and to 5.2 after 24 h (B. J. Parsons, personal communication).

In the first experiment six rats were fed the vitamin D-deficient diet for 3 weeks. Three of these were given 100 I.U. (2.5 μg, 6.5 nmol) cholecalciferol orally in olive oil; 48 h later 6 flasks of duodenal slices were prepared and incubated with 1 mM Ca (see legend to Fig. 1 for method). The Ca influx was 5.31 ± 0.17 μmol g^{-1} h^{-1} (mean ±s.e. of mean). The mean influx for three rats given olive oil without cholecal-

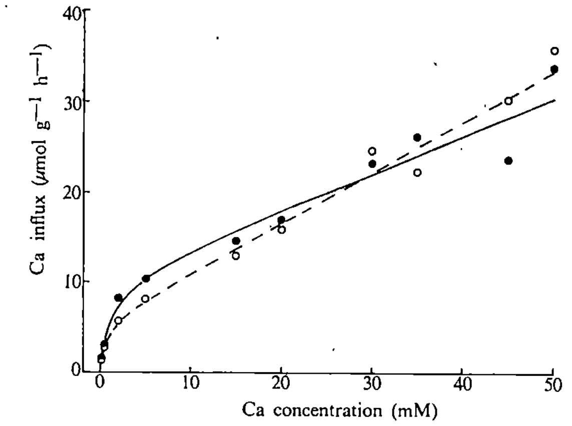

Fig. 1 Effect of cholecalciferol on Ca influx kinetics. Twelve rats were fed the vitamin D-deficient diet for 4 weeks. Six of these rats were given 100 I.U. cholecalciferol orally in olive oil, 6 given only olive oil. After 48 h duodenal slices were prepared and incubated as previously described[14]. Twelve duodenal slices were prepared from each rat, 1–5.5 cm from the pylorus. In each flask 6 slices, total fresh weight approx. 250 mg, were pre-incubated for 15 min in 8.1 ml. Ca-free medium containing 139.4 mM NaCl, 4.7 mM KCl, 4.0 mM Tris buffer at pH 7.4, and 27.8 mM glucose. Then 10.5 ml. medium was added with some Na replaced isomotically by Ca, labelled with ^{45}Ca. After 5 min further incubation the slices were washed twice with 154 mM NaCl, blotted and weighed. The uptake of ^{45}Ca was measured by liquid scintillation counting[23], to give an estimate of the unidirectional influx of Ca into the tissue[14]. Influx was across both mucosal and serosal surfaces. Each point gives influx for 1 flask of 6 slices. Points for 0.025 and 0.05 mM Ca are omitted. Curves were fitted for the expression given in the text. ●—●, Dosed with cholecalciferol; ○—○, vitamin D-deficient.

ciferol was 4.44±0.11 μmol g^{-1} h^{-1}, which was significantly less by t test ($P<0.01$).

Ca influx over a range of Ca concentrations has been analysed into saturable and linear components according to the expression[14]:

$$J=\frac{J_m[\mathrm{Ca}]}{K_t+[\mathrm{Ca}]}+P[\mathrm{Ca}]$$

where J=Ca influx, [Ca]=concentration, J_m=maximal value and K_t=half-saturation constant for the saturable (carrier) component, and P=constant for the linear (simple diffusion) component. This expression fitted the influx data well both in vitamin D deficiency and following 100 I.U. cholecalciferol in the second experiment (Fig. 1). There was a significant overall difference between the two conditions ($p=0.016$): in particular cholecalciferol increased J_m from 5.51±0.65 to 10.4±1.2 μmol g^{-1} h^{-1} ($p=0.002$), increased K_t from 0.627±0.096 to 1.22±0.18 mM ($p=0.008$), but decreased p from 0.556±0.038 to 0.402±0.047 ml. g^{-1} h^{-1} ($p=0.020$). Except at high concentrations the overall effect was to increase Ca influx, and markedly so in the physiological range: the maximal increase was 38% at 3 mM Ca.

Thus the increase in Ca influx by cholecalciferol was the result of an increase specifically in the saturable component. This component is known to relate mainly to Ca entry across the mucosal brush border[14], which was ascertained in normal, vitamin D-replete rats. The reduction in the saturable component induced by vitamin D deficiency must therefore indicate a reduction in that component of influx across the brush border. Martin and DeLuca[11] have also shown that cholecalciferol increased Ca influx across the brush border, but were unable to define Ca influx kinetics in vitamin D deficiency and did not identify a linear component. This analysis demonstrates that K_t was increased by cholecalciferol, from which it is unlikely that Ca influx was enhanced by the increased affinity of a carrier in the brush border. My results show for the first time that cholecalciferol facilitates Ca entry solely by increasing J_m, which would indicate an increase in the number of entry sites in the brush border. The biochemical correlates of these could be (i) Ca-dependent ATPase[15] (Ca entry is energy-dependent[14]), (ii) non-specific phosphatase[15–17], (iii) calcium binding protein[13] or (iv) some other entity. As in the normal rat[14], so with vitamin D action the kinetics of Ca influx correspond with the kinetics of Ca translocation across the entire epithelium: both *in vitro*[18] and *in vivo*[19] only the saturable component is increased by cholecalciferol.

In contrast, the linear component of Ca influx, P, was reduced significantly by cholecalciferol. This parameter is not specific for the mucosal surface[14], but it is possible that physiological doses of the vitamin reduce Ca diffusion across the brush border.

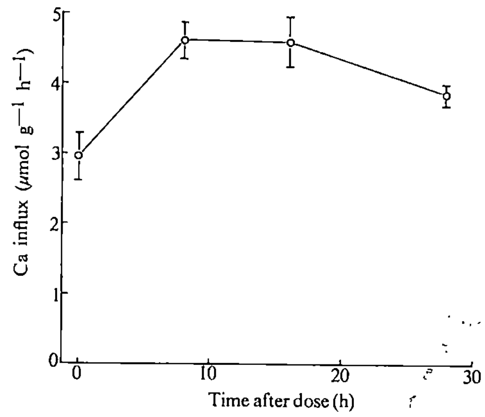

Fig. 2 Ca influx at intervals following administration of cholecalciferol. 16 rats were fed the vitamin D-deficient diet for 5 weeks. Groups of 4 rats were given 100 I.U. cholecalciferol orally in olive oil. After the times indicated 8 flasks of slices were prepared from each group and incubated with 1 mM Ca. Four control rats, shown at zero time, received no vitamin. Standard errors of the means are given.

The total Ca influx was determined at different times following the oral administration of 100 I.U. cholecalciferol (Fig. 2). The influx increased 56% by 8 h after the dose but was not further increased after 16 or 28 h. Both Ca absorption *in vivo*[20,21] and transport across everted sacs (see above) had begun to increase by about 8 h after a similar oral dose. It would seem therefore that Ca influx across the brush border increases with vitamin D at least as early as these measures of Ca absorption. The time of increase in Ca influx also corresponds closely with the appearance of 1,25-dihydroxycholecalciferol in the mucosa following administration of cholecalciferol[22].

My results support the conclusion that the primary functional response of the intestinal mucosa to 1,25-dihydroxycholecalciferol is to increase the number of sites of the carrier mechanism whereby Ca enters the cell across the brush border.

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Subcutaneous Growth of Human Tumours in Mice

CASTRO[1] reported the successful growth of human tumours in immune suppressed mice after the tumour tissue had been implanted beneath the left kidney capsule. In similar work we have used the subcutaneous route because we found this to be the most successful in our extensive investigations into tumour growth in ALS treated mice.

Female CBA mice were thymectomized at 4 weeks, whole body irradiated (900 R), 2 weeks later and reconstituted within 3–4 h of irradiation, using 1×10^7 syngeneic bone marrow cells, given i.v.

Table 1 Subcutaneous Tumour Growth

Tumour type	Percentage survival of tumours				
	1 wk	2 wk	3 wk	4 wk	5 wk
HeLa	100	100	100	53	21
Colon	100	100	100	80	
Stomach	100	100	50	38	38
Hep 2	75	75	50	50	12
Rectum	100	75	50	50	
Caecum	100	100	50	50	25
Breast adenocarcinoma	0	0	0	0	0
Secondary tumour, the primary being in the breast	70	70	70	70	
Lymph node from melanoma	100	100			

The tumours were obtained at operation and transported to the laboratory in Eagles minimal essential medium, containing 2% calf serum, 200 units of penicillin, and 100 mg streptomycin. Tumour pieces of about 2 mm were implanted subcutaneously in a ventro/lateral site over the right hip joint. In our ALS experiments we found by experience that this was the most suitable site for implantation, in view of the large tumour growths obtained, often in excess of 2–3 cm. In our present investigations we are able to report colonic tumour growths of the order of 12 mm within eleven days of implantation. This was assessed by direct palpation. Tumour types investigated are given in Table 1.

Our results clearly show that the subcutaneous route produces good tumour growth which is easy to assess by direct palpation and therefore affords a means of judging the effectiveness of treatment without having to kill the mice. We feel this method will be useful for further *in vivo* investigations of human tumours.

We thank New End Hospital and Hampstead General Hospital for the supply of some of the tumours.

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Inhibition of Pupariation in *Sarcophaga bullata*

THE classical test used as the bioassay for the insect moulting hormone, ecdysone, is based on the failure of the posterior part of ligatured larvae of *Calliphora erythrocephala* (or other fly species) to tan unless an adequate dose of ecdysone is administered. It is crucial for the success of the test to select larvae at the right stage of development, and as closely as possible to the critical time for pupariation[1]. Larvae ligatured before the critical stage of natural ecdysone release may eventually pupariate in the front part where the hormone originates, but will never do so in the hind part. Post-critically ligatured larvae have had sufficient ecdysone released into the haemolymph previous to ligature to induce tanning in the hind parts. It is evident that at this time the anterior parts must also be post-critical. In larvae ligatured at this stage the anterior parts usually contract to the puparium and tan whereas the posterior parts merely tan without change in shape. The occurrence of tanning in the posterior part alone is therefore a paradox. Fraenkel[2] along with the description of the "*Calliphora* assay" also reported that ligatured larvae occasionally tanned only in the posterior region. Several investigators have been subsequently surprised by this phenomenon of "anterior inhibition", but there has been no agreement as to its cause.

Kaplanis *et al.*[3] observed anterior inhibition in the larvae of *Musca domestica*. Langley[4] attributed anterior inhibition in the larvae of *Glossina morsitans* to an inhibitory influence of the central nervous system which in a ligatured larva is confined to the anterior part. In contrast, Price[5] hypothesized the presence of an inhibitory factor of the phenol oxidase inhibitor type, to account for a temporary inhibition of pupariation in the anterior parts of *C. erythrocephala* larvae ligatured between segments 8 and 9. Fraenkel and Zdarek[1] reported that in different species of flies the percentage of anterior inhibition is different. In the study reported here, with *Sarcophaga bullata*, it varied, according to the condition of the experiment, between 0 and 80%. Chang[6] observed a 96.9% anterior inhibition in *Lespesia archippivora* that had been in his view ligatured "pre-critically", and suggested that, contrary to common notions, ecdysone was produced in the posterior parts only. The injection of ecdysone into such anterior parts did not, however, induce tanning. In the absence of evidence about the pre-critical nature of his larvae we suggest that they were indeed ligatured post-critically and showed the same effect of anterior inhibition observed in the other species.

Our studies now confirm Fraenkel's original view[2] that the inhibition of pupariation in post-critically ligatured larvae was

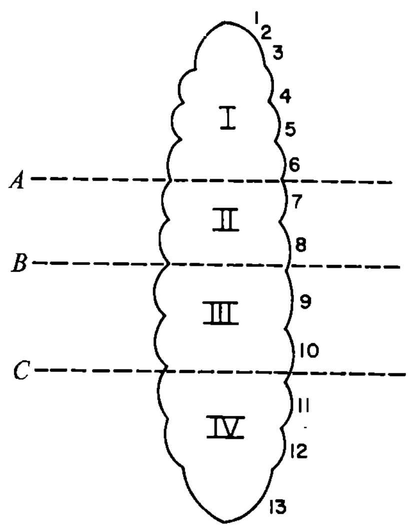

Fig. 1 Diagrammatic figure of larva of *S. bullata* showing positions of ligature.

caused by lack of oxygen. The experiments described here were carried out with *S. bullata* larvae, in the post-critical state, about 2–3 h before pupariation, when the region around the spiracles has begun to tan.

The anterior inhibition of post-critically ligatured larvae could conceivably be due to the inactivation of ecdysone[7]. Karlson and Bode[8] reported the presence of such an inactivating enzyme in *C. erythrocephala* larvae. The rate of inactivation in whole larvae was greater than that in posterior parts of pre-critically ligatured larvae. Does the anterior inhibition result from the more rapid inactivation of ecdysone in the anterior parts? Massive doses of β-ecdysone (1 and 2 μg, that is, 30 to 60 *Sarcophaga* units[9]) injected into each of fifteen inhibited anterior ends in each concentration within 1 h of tanning of the posterior ends, did not result in the subsequent tanning in any of them, ruling out the inactivation of ecdysone as the probable cause.

Langley's hypothesis[4] of the central nervous system inhibiting the action of ecdysone in response to stimulation of surface sense organs was not supported by any evidence. Tetrodotoxin had been shown by Zdarek and Fraenkel[10,11] to cause complete paralysis and a complete or substantial block of nerve conduction without blocking pupariation in *S. bullata* larvae (0.1 μg per larva or considerably less). The injection of 0.05 μg of tetrodotoxin into anteriorly inhibited ends did not release the inhibition of pupariation, suggesting the absence of a significant inhibitory stimulus from the sense organs to the central nervous system.

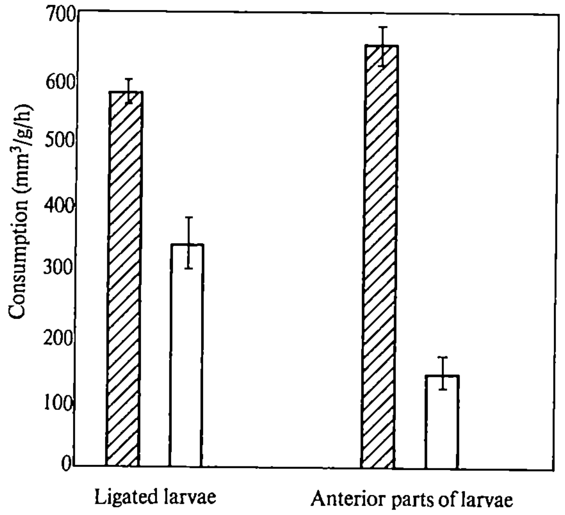

Fig. 2 Comparison of oxygen consumption in anteriorly inhibited and uninhibited whole ligatured larvae and isolated anterior parts. Bars indicate one standard error on either side of the mean value. ▨, Uninhibited (tanned); ☐, anteriorly inhibited (untanned).

Price[5] observed that in *C. erythrocephala* larvae ligatured between segments 6 and 7, 8 and 9, and 11 and 12, segments 7 and 8 alone remained untanned. This he attributed to the presence of an inhibitory factor, of a phenol oxidase inhibitor type, in this region. In repeating Price's experiments, with *S. bullata*, the ligatures were made so as to minimize injury to the larvae, that is, not too tightly. In the first experiment the larvae were ligatured at positions *A*, *B* and *C* (Fig. 1) in that order The results were identical with those of Price; only region II remained untanned when observed 6 h and 25 h later. However, when the ligatures were applied in the reverse order of *C*, *B*, *A*, only region III remained untanned. When ligatured in the order *A*, *C*, *B*, or *C*, *A*, *B*, both regions II and III remained untanned. Each of these sets of experiments was carried out with twenty-five larvae, with invariably the same result. Thus the inhibition always occurred between the first two ligatures applied irrespective of their positions. In no case did regions I and IV, which bear the spiracles, show inhibition. (The expression of anterior inhibition is variable.) We found that the first two ligatures can be tightened very firmly without rupturing the body wall, whereas if the third ligature was tightened to the same degree it would cause a rupture with haemolymph flowing out. Thus the third ligature was never applied as tightly as the first two, and

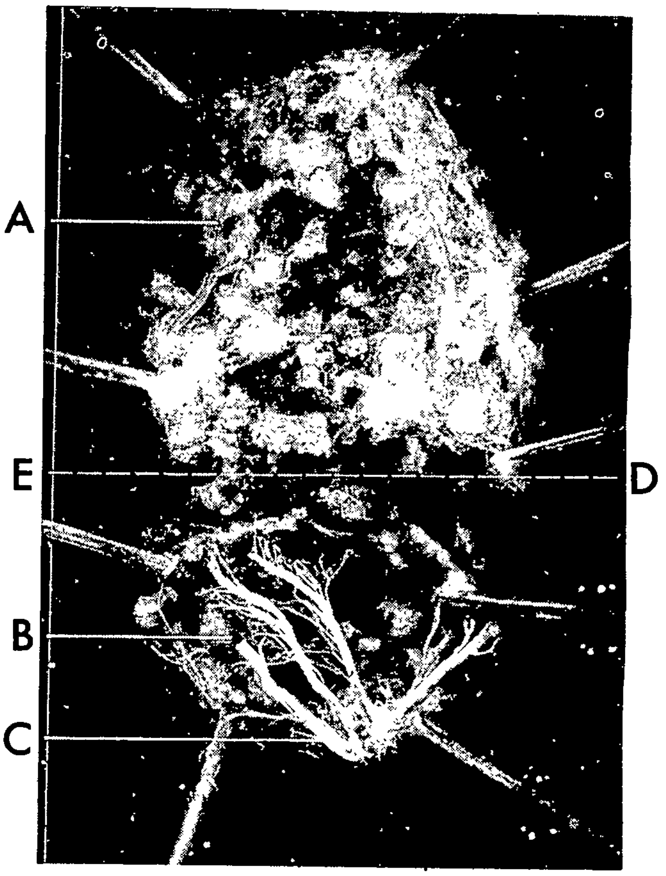

Fig. 3 Dissection of a ligated larva, exhibiting anterior inhibition of pupariation. A, Tracheae filled with haemolymph; B, plug of coagulated haemolymph; C, air-filled tracheae; D—E, position of ligature.

did not cause a disconnexion of the tracheal trunks. The invariable tanning of the two regions bearing the spiracles and the inhibition of the spiracle-less regions of the larvae between the first two ligatures to be applied indicates that inhibition is caused by the cutting off of the tracheal supply of the air through the spiracles. These experiments were repeated with *C. erythrocephala*, with the same results. Therefore the need to postulate an inhibitory factor, localized in a particular region, does not arise.

If the inhibition is caused by the cutting off of the tracheal supply of air, it would follow that the larvae showing inhibition would consume less oxygen than those that do not show it. Oxygen consumption was measured with a Scholander respirometer (Mark Co.), at 26° C. Measurements were made at hourly intervals for 4 h from the time of ligature, and the volume of oxygen consumed per hour per gram wet weight was computed. Where the oxygen consumed by the anterior parts only was measured, the posterior parts were cut off after ligature between segments 8 and 9. The results (Fig. 2) are mean values for at least ten specimens tested separately. Inhibition in a particular larva cannot be predicted at the time of ligation and can be observed only 2–3 h later.

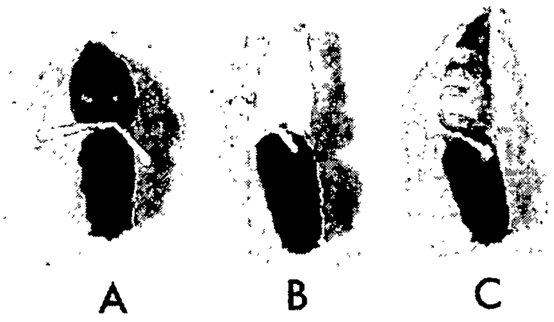

Fig. 4 Effect of oxygen on anterior inhibition of tanning. A, Ligatured larva with no anterior inhibition; B, ligatured larva with anterior inhibition; C, ligatured larva with anterior inhibition, subsequently subjected to a stream of oxygen.

The volume of oxygen consumed by the ligatured larvae which subsequently showed anterior inhibition was approximately 70% of that of larvae that did not show it. This is because of the decreased uptake of oxygen by the inhibited anterior ends. Inhibited isolated anterior ends consume only about 25% of the oxygen consumed by the uninhibited anterior ends (Fig. 2). This reduction in oxygen uptake closely resembles that in sections of *C. erythrocephala* without spiracles[12].

Our experiments have shown that spiracle-less regions firmly isolated by ligatures do not tan. The measures of oxygen consumption showed a startling decrease in anterior ends destined to be inhibited, but these anterior parts bear the prothoracic spiracles. In *S. bullata* larvae, like those of many fly species, only the prothoracic and the last abdominal spiracles are functional. The anterior spiracles are, however, far smaller. Is the decreased oxygen consumption a direct result of the cutting off of the tracheal air supply in these cases too, and if so, how is it caused?

Dissection of anterior parts showed that internal wounding caused the inhibition. The tracheae in the inhibited specimens were completely severed at the point of ligature and did not appear silvery, but instead were filled with a liquid (Fig. 3). They appeared colourless at the time of dissection, and gradually acquired a black colour reminiscent of the darkening of haemolymph when exposed to air. The tracheae of the uninhibited anterior ends remained silvery due to the air within the lumen. Here too the tracheae were severed in some, however the severed ends were covered with a black plug of tissue (as seen in the posterior part of Fig. 3). Thus it seems that the decreased oxygen consumption in the inhibited anterior ends comes about because the tracheal lumina is flooded with haemolymph, thus preventing air from reaching the tissues.

To confirm this view, anteriorly inhibited larvae in which the tracheae are blocked were subjected to a steady stream of oxygen for 6 h. These anterior ends which normally never tan showed tanning to a certain degree after this treatment (Fig. 4). This shows that the higher oxygen uptake allowed some tanning to take place.

Thus, inhibition of tanning in post-critically ligatured fly larvae can be produced either by eliminating the spiracles, or by abolishing tracheal function as a result of the spiracles being filled with liquid. It is interesting that in tsetse flies the anterior spiracles are not open to air which readily explains the 100% inhibition[4] of tanning in the anterior parts. It is known that tanning of the puparium depends on oxygen reaching it from inside through the tracheae, since too little penetrates through the cuticle from outside[2,12]. The situation is changed radically in pure oxygen when oxygen consumption in the absence of spiracles is at least three times that in air[12] and sufficient oxygen can penetrate to the sites of action in the anteriorly inhibited specimens to cause considerable tanning. Anterior inhibition therefore results from a lack of oxygen caused by the injury to the tracheal system during ligature. The extent of this injury depends on the tightness of the ligature, and is different in different species.

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Tension Receptor Reflexes in the Walking Legs of the Crab *Cancer pagurus*

THE decapod crustaceans have many advantages for the study of the neuronal mechanisms controlling simple behaviour[1-3]. Numerous authors have investigated the functions of the walking legs, but models of the control mechanisms have been presented in terms of central programmes and reflexes related simply to the positions and movements of the joints[4,5]; little attention has been paid to the control of tension in the chief working muscles. It has been shown, however, that the tension of some of these muscles is monitored by sense organs[6]. We present here some initial findings on the reflexes produced by input from these tension receptors and the integration of such reflexes in the intact animal.

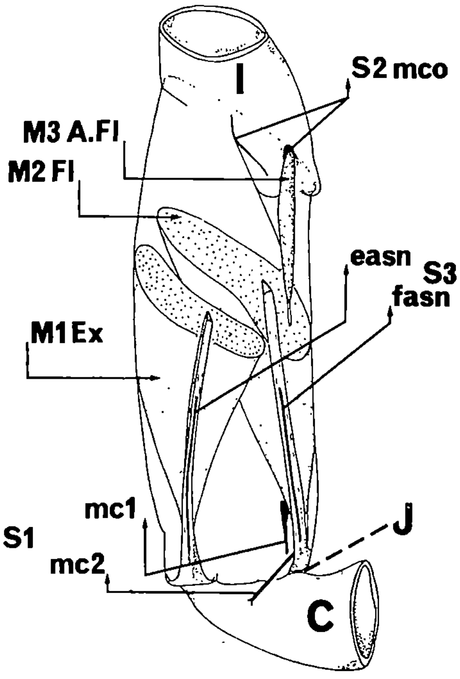

Fig. 1 The organization of the muscle (M1–3) and sensory (S1–3) systems controlling the M–C joint (J) of the first right walking leg. All the structures lie in the merus which is seen from the anterior surface lying between the ischium (I) proximally and the carpus (C) distally. The extensor (M1 Ex) lies on the anterior side of the segment and attaches to the carpus dorsally. It is innervated by two excitatory neurones and one inhibitory motor neurone. The flexor (M2 Fl) lies on the posterior side of the segment and attaches ventrally to the carpus. It is innervated by four excitatory neurones and one inhibitory motor neurone. Further, a small accessory flexor muscle (M3 A.Fl) attaches by way of a tendon to the distal end of the flexor apodeme. The simple chordotonal organs (S1 mc1, mc2) lie ventrally near the joint and are attached to the flexor muscles. For clarity the distal part of the accessory flexor muscle and the sub-groups of the myochordotonal organ (S2 mco) are not shown.

The investigation was carried out on the merus carpus (M–C) joint of *Cancer pagurus*. The organization of this joint (Fig. 1) is very similar to that of *Cancer magister*[7]. It allows almost 180° of movement in the dorso-ventral plane and is controlled by two major muscles which lie in the merus. Three types of sense organ are known at M–C. The simple chordotonal (S1) organs monitor joint position and movement, and generate resistance reflexes which oppose imposed (passive) joint movements[8]. It is curious therefore, that these reflexes should operate during walking to oppose ongoing (active) movements. In the propus-dactylus joint resistance reflexes do not occur during undisturbed walking on even surfaces (that is, where the possibility of imposed load variations from cycle to cycle has been eliminated), but the mechanism governing this inhibition is unknown[4]. At M–C it is thought that in such circumstances the resistance reflexes operate only at extreme joint positions[5]. The complex myochordotonal organ (S2) monitors joint movements but it can also be set, without joint movement, by output to the accessory flexor muscle from the CNS. During movement the receptor generates subsidiary resistance reflexes. Its principal role, however, is thought to be in a complex set of reflexes which stabilize joint resting position[5].

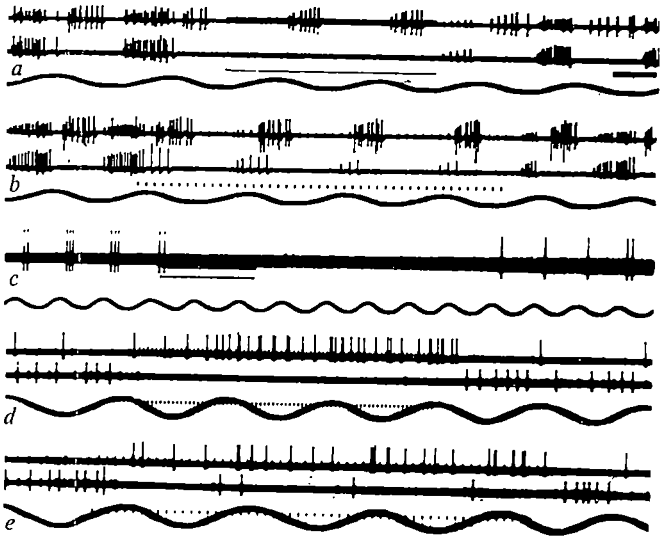

Fig. 2 Effects of stimulations of apodeme sensory nerves on resistance reflex motor activity. All stimulations were series of pulses of length 0.5 ms at low stimulus strengths. Each set of traces is described from top to bottom. *a*, Flexor plus extensor myogram, extensor myogram, stimulus of 50 pulses s^{-1} (line), movement of merus–carpus joint with flexion, an upward deflexion and extension downwards; *b*, as in *a*, but stimulus 5 pulses s^{-1}; *c*, extensor motor nerve, stimulation at 20 pulses s^{-1}, joint movement with flexion downwards; *d*, extensor myogram, flexor myogram, joint movement with extension upwards, stimulation at 20 pulses s^{-1}; *e*, as in *d*, but 10 pulses s^{-1}. Time mark is 1 s in *a* to *c* and 0.5 s in *d* to *e*.

The tension receptors (S3) are associated with the extensor and main flexor apodemes. The sensory fibres run with the respective motor nerves proximally but separate distally to form distinct apodeme sensory nerves. Each apodeme sensory nerve carries numerous axons from bipolar cells whose dendrites run on the surface of the apodemes. Little activity was recorded in either sensory nerve when the joint was moved passively with low tension in the relevant major muscles. When the joint was fixed and the motor supply to a muscle stimulated, sensory responses were recorded from its apodeme sensory nerve at tensions well within the normal range observed in intact animals. All the units sampled responded with an increase in activity to increasing tension but other responses could be present in small neurones[6].

In our experiments crabs were placed in a clamp with a platform on which the merus of the first right walking leg was fixed with the anterior surface exposed for dissection (the levator muscle apodeme of the leg was cut to prevent autotomy). Myograms were recorded from both major muscles with implanted insulated copper wire electrodes, and after a simple dissection, platinum wire electrodes were used to stimulate the extensor apodeme sensory nerve and to record from branches of the extensor motor nerve. Alternatively, by dissecting further into the meropodite the platinum wire electrodes could be used to stimulate the flexor apodeme sensory nerve and to record from branches of both flexor and extensor motor nerves.

Movements of the carpus were made with a potentiometer-monitored micromanipulator. Conventional methods of stimulation, amplification, display and photography were used.

Stimulation of the extensor apodeme sensory nerve at very low voltage (that is, activation of only large receptor neurones) effectively inhibited any spontaneous extensor motor activity. Extensor motor activity produced by evoking resistance reflexes (initial part, Fig. 2*a*) was also inhibited by stimulation of the extensor sensory nerve. Total inhibition was always obtained at 50 pulses s^{-1} (Fig. 2*a*), but the inhibition could be reduced by lowering the stimulus frequency (Fig. 2*b*). After a stimulus, inhibition could persist for many seconds (Fig. 2*c*). The effects on the antagonistic flexor motor neurones were more varied. In some examples there was little change in flexor activity during stimulation of the extensor sensory nerve (Fig. 2*a*, *b*) but more often an excitation was observed. We suspect that other factors (for example, the flexor apodeme sensory input) are involved in the antagonistic motor response. Stimulation of the flexor apodeme sensory nerve during passive movements of the carpus produced effects symmetrical to those observed for the extensor sensory nerve. At low stimulus strengths inhibition of the flexor motor activity was obtained (Fig. 2*d*) and this effect could be modulated by changes in stimulus frequency (Fig. 2*e*). Again, more variable results were obtained for the antagonistic (extensor) motor neurones but most often an excitation was observed (Fig. 2*d*, *e*). No significant changes in any of the responses to low stimulus strength activation of the apodeme sensory nerves were observed after destroying the myochordotonal organ receptor system by removal of the accessory flexor muscle.

Stimulation of either apodeme sensory nerve at much higher stimulus strengths produced, after a short latency, a strong discharge in both extensor and flexor motor neurones. In all experiments the identities of the stimulated and recorded nerves were subsequently verified by staining with methylene blue. Additionally, a series of control experiments confirmed that the results were obtained from apodeme sensory input to the CNS.

The crab M–C joint has now been shown to have three different types of sensory receptor, each of which can be divided into at least two distinct sense organs. In view of this complexity findings of no malfunction after ablation of single sense organs in such situations do not necessarily mean that the ablated receptor has no function in the intact animal[9,10]. The widespread occurrence of tension receptors in the decapods[6], and the possibility of hysteresis in the responses of crustacean muscles to motor output[11], also mean that for this group the regulation of muscle tension can no longer be ignored in models of neuronal control mechanisms governing simple behaviour.

The fact that the apodeme sensory input, which is dependent on muscle tension, can profoundly modify the resistance reflexes due to the chordotonal organs, may explain the inhibition of resistance reflexes during undisturbed walking on even surfaces, although an entirely central inhibition would seem to be a more economical mechanism. We suggest, however, that during normal walking on uneven surfaces the crab is as likely to encounter resistance to active movements as imposed (passive) joint movements. Thus reflexes generated by tension receptors (when an active movement is prevented) could be just as important as resistance reflexes in the intact animal. Tension receptor reflexes are clearly of some importance in cockroach walking[12].

The demonstration of a negative feedback loop from sense organs in series with the meropodite working muscles suggests that the apodeme receptors are analogous to vertebrate Golgi tendon organs. We would be cautious of a direct analogy because the effects of higher stimulus strength activation of the sensory nerves suggest that the apodeme sense organs also contain elements which evoke strong excitatory reflex output to the innervated muscle. More sophisticated experiments will be required to determine the nature of any such positive feedback elements. The same holds for the more complex effects that we have observed for the antagonistic muscles in these experiments. Our findings nevertheless show that the sensory array at the crab M–C joint is more comparable to the vertebrate system than was previously thought, and thus probably more useful for the study of general principles.

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Trend towards Earlier Menarche in London, Oslo, Copenhagen, the Netherlands and Hungary

Brundtland and Walløe[1] have reported that the trend toward earlier maturation in children, as evidenced by age at menarche, has stopped, at least for the present. Using probit estimates in a *status quo* inquiry they gave the mean ages as 13.27 yr in 1952 and 13.24 in 1970. Both results were based on large samples of Oslo schoolgirls.

Here I report a similar finding in London schoolgirls. In a large-scale survey[2] made in 1959 in randomly selected London schools the mean, estimated by probits, was 13.05 ± 0.02 yr. A survey made between November 1966 and February 1967 (unpublished data), using a similar sample, gives a mean of 13.02 ± 0.03 yr. This figure relates only to those girls (approximately 7,000) between the ages of 9 and 16 whom the health visitor questioners had noted as "European" as opposed to "non-European". Because there were fewer non-Europeans in 1958 than in 1966 this may make the two samples more comparable. Inclusion of non-European girls, however, alters the value only trivially.

The previously well marked trend, therefore, seems to have stopped in London and Oslo. This is not (? yet) the case in all parts of Europe. Two other studies, comparable in both numbers and method with those cited, show that in Holland[3] between 1955 and 1965 the mean age decreased from 13.6 yr to 13.4 yr, and in West Hungary[4] between 1960 and 1965 it decreased from 13.27 ± 0.07 to 13.13 ± 0.01 yr. A slightly less reliable sample of Copenhagen girls showed a decrease from 13.8 ± 0.03 (ref. 5) in 1950 to 13.2 ± 0.2 in 1964 (ref. 6). Some of the best series are illustrated in Fig. 1).

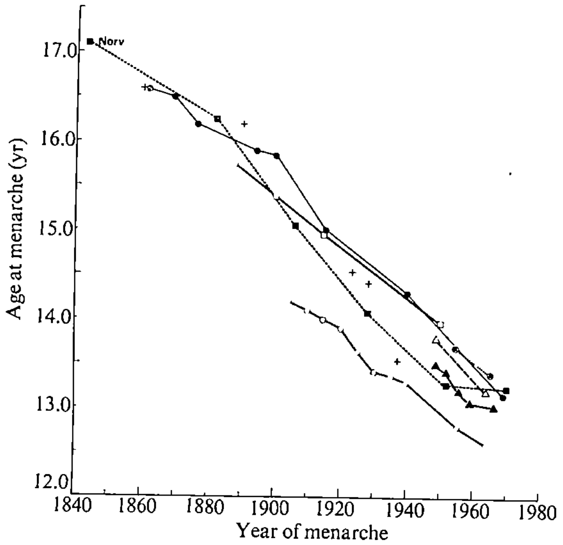

Fig. 1 Trend in menarcheal age in some European countries and the United States 1840–1970. Redrawn, with additions, from Tanner[7]. △, Denmark; ●, Finland; +, Germany; ⊙, Holland; ■, Norway; □, Sweden; ▲, United Kingdom; ○, United States.

I suggested earlier that the trend would continue until North European means reached somewhere near the value for Americans[8] (about 12.7 yr), Italians[9] (about 12.6 yr), or even perhaps Chinese[10,11] growing up in economically similar circumstances (about 12.4 yr). It looks as if this expectation will prove to be wrong; in the next decade Dutch, Danes and Hungarians may join the standstill of the Atlantic fringe.

The data on London girls will be published in detail elsewhere[12]. I acknowledge the initiative and cooperation of the Inner London Education Authority and the support of the Nuffield Foundation.

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Outbreeding and Inbreeding in a Zinc–Lead Mine Population of *Armeria maritima*

ANTONOVICS[1] and Lefèbvre[2] have shown that self-fertility occurs in zinc–lead mine populations of normally incompatible species (*Agrostis tenuis, Anthoxanthum odoratum, Armeria maritima*). In *Agrostis* and *Anthoxanthum*[1] this change to inbreeding is suggested to be an adaptation to reduce gene flow from plants growing adjacent to the mine that do not tolerate metals. In *Armeria*[2] there is no surrounding non-tolerant population and self-fertility has been assumed to be a mechanism usually found in colonizing species which enables a reliable production of seeds in colonizing situations where individuals are both few and sparse[3,4].

Table I Number of A-types and B-types in Ten Samples Collected from the Population of Plombières

Samples	A-type	B-type	$\chi^2_{50\,:\,50}$	$\chi^2_{48\,:\,52}$
1	461	383	5.54	12.49
2	127	123	0.06	0.79
3	138	122	0.98	2.60
4	272	267	0.02	1.25
5	194	201	0.16	0.16
6	147	181	3.64	1.22
7	47	53	0.36	0.04
8	46	54	0.64	0.16
9	54	46	0.64	1.44
10	51	49	0.04	0.36
Total	1,527	1,479	12.08	20.51

In these experiments inbreeding was artificially induced by enclosing inflorescences in paper bags. This method gives data on potential self-fertility but does not give any idea about the efficacy of self-fertility in the mine population. Selfing obtained by artificial methods can disappear in natural conditions[5]. I report here results on outbreeding estimates established for a population of *Armeria* collected from the mine site of Plombières (Belgium). Mating-type genes were used. Reproduction, in European populations of *Armeria*, is controlled by a dimorphic self-incompatibility system which breaks down to monomorphic self-compatibility in Arctic and New World races[6]. In European populations, half the individuals are heterozygous with reticulate pollen and smooth stigmas (A-type *AC*/*ac*) and the other half are homozygous with punctate pollen and papillous stigmas (B-type, *ac*/*ac*). Pollen type and stigma type are determined by the supergene AC (ref. 6). Fertile combinations are only possible between A-type and B-type so that the 50:50 ratio is maintained through generations.

Table 2 Statistical Analysis of Data in Table 1

Source	*d.f.*	$\chi^2_{50\,:\,50}$	$\chi^2_{48\quad 52}$
Total	10	12.08	20.51 *
Pooled	1	0.69	9.65 †
Interaction	9	11.39	10.86

* $P<0.05$. † $P<0.01$.

If inbreeding occurs in a 50A/50B population there will be a predominance of B-type, as found by Baker is a population collected in the Shetland Islands, in which some individuals were self-fertile.

Table 3 Results of Progeny Tests in Three Samples of the Population of Plombières

Samples	Plombières 1		Plombières 2		Plombières 3		Total	
Parents	B-type	A-type	B-type	A-type	B-type	A-type	B-type	A-type
Number of parents tested	18	8	14	19	19	15	51	42
Offspring B	383	123	122	267	201	181	706	571
Offspring A	451	127	138	272	194	147	783	546
Frequency of B	0.460	0.492	0.470	0.495	0.509	0.551	0.473	0.511
Frequency of A	0.540	0.508	0.530	0.505	0.491	0.449	0.527	0.489
Outbreeding coefficient (λ)	1.024		1.020		1.093		1.049	

If all the individuals in an initial 50A/50B population become completely self-fertile a new equilibrium will be established after a few generations at 25A/75B. As the mean self-fertility in the Plombières population is approximately 7%, a 48A/52B ratio must theoretically be reached.

To test this disequilibrium, population samples were scored for A and B types (Tables 1 and 2). Statistical analysis shows clearly that there is no significant deviation from the 50A/50B ratio and that the results are statistically incompatible with a 48A/52B ratio. This suggests that inbreeding is not occurring at the present time in the population.

In addition, progeny tests were carried out on three samples of the population (Table 3) to estimate the outbreeding coefficient according to the method of Vasek[7]. Two equations are solved:

$$\lambda p = DR \qquad (1)$$

frequency of dominants in the progeny of recessive homozygous.

$$\tfrac{1}{4}(1+\lambda q-\lambda p)=RH \qquad (2)$$

frequency of recessives in the progeny of heterozygous where λ=frequency of outcrossing, p=frequency of dominant gene, q=frequency of recessive gene.

Because of the breeding system in *Armeria*, where $DR=0.50$ and $p=0.25$, λ has a theoretical value of 2; this value must be divided by two as only half of the possible crosses exist. Table 3 shows that in the three plots λ has a value of approximately 1.

On the other hand, if inbreeding occurs in the population, some A plants should be homozygous dominant (AA) and produce only A-type progeny in the progeny test. If total inbreeding occurs, the frequency of AA in the A-type progeny will be 0.50. The potential amount of inbreeding in the population (7%) leads to the estimate that approximately 4% of AA plants must arise in the population. In our experiment all the A-plants tested give A and B-types, without exception, in their progeny. This is additional evidence against self-fertility in this population.

It is thus obvious that outbreeding is complete at the present time in the mine population of Plombières although some potential self-fertility has been shown artificially.

The adaptive significance of self-fertility in mine populations must therefore be reviewed. In Plombières nearly all plants grow at a high density so that there is no spatial barrier to outbreeding. It is also probable that pollen competition favours foreign pollen. In this situation self-fertility has no adaptive value. But if new areas on the mine are available for colonization, self-fertility could be important. To test this assumption progeny tests need to be carried out on plants isolated during a colonization.

In fact, self-fertility seems to be the relic of an adaptive character which has no effect now in the dense mine population studied here. In *Agrostis* and *Anthoxanthum* more information is needed on the efficacy of self-fertility in the mine population compared with gene flow from the surrounding population because self-fertility could be just an adaptation to initial colonizing stage as in the case of *Armeria*.

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New Endocranial Values for the East African Early Hominids

THIS is a preliminary report on research carried out during 1971 and 1972 on the endocranial values of some East African hominids from Olduvai Gorge, Tanzania, and East Lake Rudolf, Kenya. The cranial remains suitable for the reconstruction of volumetrically accurate endocranial casts are as follows: from Olduvai Gorge, hominids OH 7, 9, 12, 13 and 24; from East Lake Rudolf, KNM-ER 732 and 406. Each set of hominid remains required its own reconstructive procedures; a full description of each is unnecessary here but will be published elsewhere. In this communication I report only the results and make some preliminary observations as to their significance. Table 1 shows the cranial capacities found for each hominid. Not all of these results should be equally regarded; some results are only approximations but others are stated with more confidence.

The cranial capacities for OH 12, 13 and 24, are based on partial plasticine reconstructions added to accurate plaster casts of the interior of the cranial fragments. OH 12 and 13 required considerable addition of plasticine, particularly in the frontal region.

OH 24 required correction of the distortion of the existing endocranial cast as produced by Mr Ron Clarke. Two coronal sections were made in a plaster cast of the original reconstruction as most of the distortion seemed to be in the vertical plane. The sections roughly divided the endocast into thirds which were then rotated so that the dorsal segments left a small gap on either side of the central portion. Some small degree of reduction of the biparietal width was necessary for the distortion has clearly enlarged this dimension. Finally, the endocast of the brain stem was carved out with a diamond rotary saw, and was correctly oriented in a more posterior position.

All of the reconstructions except KNM-ER 406 were coated with preservative and immersed in water to give the final displacement volume[1]. OH 7, based on the juvenile parietals, and estimated at 687 cm^3 by Tobias[2,3] was not reconstructed, but "fitted" over existing full endocasts. The endocast of OH 16 was not reconstructed as the skull reconstruction is not good enough, in my judgment, to give an accurate internal surface replica.

Table 1 Volumetric Results

Specimen	Taxon	Cranial capacity (cm^3)	Method
OH 9	*Homo erectus*	1,067	Latex mould + plasticine
OH 12	*H. erectus* (?)	727	Plaster cast + plasticine
OH 13	*H. habilis*	650	Plaster cast + plasticine
OH 24	*H. habilis*	590	Plaster cast + plasticine + correction
KNM-ER 406	*Australopithecus robustus boisei* (male)	510	Volume formula + measurements
KNM-ER 732	*A. robustus boisei* (female)	506	Latex mould + plasticine

Average for *H. habilis*, including 650 cm^3 value for OH 16, and 687 cm^3 for OH 7, = 637 cm^3.

(s.d. = 64 cm^3, assuming coefficient of variation = 10%.)

Average for robust australopithecines, including OH 5 at 530 cm^3 and SK 1585 at 530 = 517 cm^3.

Average for gracile australopithecines[11] ($n=6$) 442 cm^3.

OH 9 and KNM-ER 732 were endocast directly from the originals using rubber latex; later small amounts of plasticine were added before determination of the total volume after first checking the dimensions of the endocast moulds against the originals. The differences in measurements were under 1%. In the case of OH 9, two reconstructions were made; the second followed Dr Alan Walker's skilful excavation of matrix from the middle and anterior cranial fossae. This permitted an unequivocal determination of the cranial volume. Only minimal modification was needed at the base and some plasticine was added to the parietal segments, particularly on the right side. Only the second reconstruction is given here. KNM-ER 732, comprising the right half of a presumably female robust australopithecine[4], was endocast directly with latex stabilized with plaster; plasticine was added to form the missing occipital and cerebellar lobes. The small fragment of the occipital bone that shows part of the right occipital condyle, the position of the hypoglossal canal and an accessory vein, was utilized to reconstruct the region of the foramen magnum.

The volume of the KNM-ER 406 skull was estimated by using the formula[5] $V=f\ \frac{1}{2}(LWH)+\frac{1}{2}(LWB)$, where f was obtained by using the average fs from OH 5 and from the new SK 1585 from Swartkrans[6,7]. The dimensions of length (L), width (W), breadth (B) and height (H), were taken externally; estimates of bone thickness based on both KNM-ER 406 and OH 5 were subtracted from the external measurements. These dimensions should closely approximate the true internal endocast dimensions of KNM-ER 406.

Of all the reconstructions made, only OH 12 could show considerable error, because it has the most plasticine addition. This involves all of the frontal lobe and the temporal lobe. The frontal and temporal reconstructions were made by placing the right temporal fragment, showing part of the foramen ovale and the middle meningeal artery, in correct relation with the remaining main parietal fragment. The bregmatic and frontal portions were similarly placed in the best position in relation to each other and the remaining endocranial reconstruction.

The results show (Table 1) that the volumes for the Olduvai hominids are either the same as or very near to, those given by Tobias[2,3] whose estimates were made by the method of partial endocasts. Table 2 gives some of the statistical results using Student's t test for small samples[8], using standard deviations based on the assumption of a coefficient of variation of 10%. Naturally the small sample sizes involved make this very speculative. Nevertheless, even with a small sample size and large standard deviations, the values of "t" are still very high. Assuming that the mean values are reasonably close to the true means, the values of "t" would become considerably higher as the sample size increased, and it is doubtful whether the standard deviations would go beyond a figure giving more than a 10% coefficient of variation. OH 7 and 16 have been included as part of the habiline sample, using the values given by Tobias[2,3]. Although the value of 650 cm^3 for OH 16 may be doubtful in view of the fragmentary nature of the skull, it is unlikely that it is grossly inaccurate if the large size of the frontal portions and the associated dentition are considered. The high value given for OH 7 (687 cm^3) does not in any way seem extreme because no acceptable alternative ways of orienting the parietals could be found[9]. Furthermore, the parietals of OH 7 more than cover the completed OH 13 parietal regions on the reconstructed endocast (which gave a value of 650 cm^3) and came closest to fitting the 727 cm^3 reconstruction for OH 12 thus giving added support to the value found earlier by Tobias.

Table 2 Statistical Results

Student t test (two tailed)[8]			
	t	P	
(1) "Gracile" vs "robust" australopithecines*	2.46	<0.02	<0.05
(2) "Robust" australopithecines vs *H. habilis*	2.91	<0.02	<0.05
(3) "Gracile" australopithecines vs *H. habilis*	5.76	<0.001	
(4) "Gracile" and "robust" australopithecines vs *H. habilis*	5.38	<0.001	

* With $n=6$ for both groups, $t=3.56$ $P<0.01$ $>0\ 0011$

While the study of the morphological attributes of these endocasts is still in progress, the volumes and endocast shapes support the concept of a more advanced hominid population both distinct from *Australopithecus africanus*, *Australopithecus robustus* and *Homo erectus*, namely *Homo habilis*.

It would be unwise at this time to draw conclusions about the relationships between the fossils on the basis of endocast shapes until they have been more thoroughly studied. The KNM-ER 406 cranium is extremely similar to the cranial remains of OH 5; both gross observation and external measurements indicate that KNM-ER 406 is slightly smaller than OH 5. The appearance of the collateral vein in the occipital portion of KNM-ER 732, the overall shape and small size of the endocast, as well as the more gracile nature of the skull all suggest that KNM-ER 732 is most likely to be a female robust australopithecine skull[10]. The value of 506 cm^3 for ER 732 represents 95% of the 530 cm^3 values for OH 5 and for SK 1585, a value also consistent with sexual dimorphism as an explanation of the difference.

The low value of 732 cm^3 for endocranial volume of OH 12 (VEK IV), obtained from such a high geological level (Bed IV) in relation to the 1,067 cm^3 value for OH 9 (Upper Bed II), raises some interesting questions. Are these values simply the extremes of a range? Is OH 12 a remnant of the earlier *Homo habilis* taxon? Is it simply a small female *Homo erectus*? On the basis of the skull alone, OH 9 was certainly a large and very robust hominid. The occipital and other skull fragments of OH 12 are very thick, and one fragment, that of the left petrous temporal, does not seem to belong with the other fragments attributed to OH 12, but must have come from a somewhat larger, second individual. Even accepting the OH 9 as a large male from the upper end of the range and OH 12 as a small female, there still remains a very considerable difference, and also a large hiatus between the Bed II habilines and OH 9. This, plus the announcement[10] of a new skull, with an estimated cranial capacity of 800 cm^3, dating from a 2.6 m.y. level from East Rudolf, would make any simple anagenetic explanation of hominids, existing as either single lines or types, as extremely speculative and unlikely.

I am indebted to the late Dr Louis, Dr Mary Leakey, Mr Richard Leakey, Dr John Harris and all of the staff at the Centre for Prehistory and Palaeontology in Nairobi, Kenya, for their kindness and cooperation, and to Dr Alan Walker for his help and advice in making these reconstructions. This work was supported by a grant from the National Science Foundation.

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Microbial CO_2 Fixation in the Anoxic Zone in a Volcanic Acid Lake

MEASUREMENT of CO_2 uptake using the ^{14}C technique[1] in a volcanic acid lake Katanuma, in northwestern Japan, showed that microorganisms assimilate CO_2 in dark and anoxic conditions even though the *p*H of water is as low as 1.8.

Katanuma is a volcanic acid lake with an area of 0.1 km^2 and a maximum depth of 25 m. The *p*H of water ranges from 1.8 to 2.0, and the bottom is covered with sulphur precipitated from lake water by oxidation of H_2S. Volcanic gases, H_2S and CO_2, are supplied from fumaroles at the lake bottom. The lake water circulates from surface to bottom in spring and autumn, and stagnates in summer and winter when the lake is covered with ice. In summer (July 1972), the anoxic layer occurred below 4 m, and a slight amount of H_2S was detected in it but, in winter (January 1970), under ice cover, dissolved oxygen was found only down to a depth of 0.5 m, and an accumulation of H_2S was observed below 1 m depth. The electrode potential value calculated for the anoxic water of 20 m depth in winter was −27 MeV.

The CO_2 fixation was determined by the ^{14}C technique[1], and CO_2 concentration in water was measured with an infrared CO_2 analyser[2]. Results showed that in the dark, in summer, CO_2 fixation of the oxic layer was very low although the photosynthetic CO_2 fixation was rather high (8.92 mgCi m^{-2} day^{-1}). On the other hand, in the anoxic layer there was no indication of photosynthesis, but a considerable amount of CO_2 fixation in dark (19.08 mgCi m^{-2} day^{-1}) was observed. In winter a very low photosynthetic activity was determined just below the ice, while a measurable amount of CO_2 fixation in dark (1.24 mgCi m^{-2} day^{-1}) was obtained from the anoxic layer. The presence of microorganisms was observed microscopically in samples from anoxic layer, but taxonomic and physiological studies have not yet been completed.

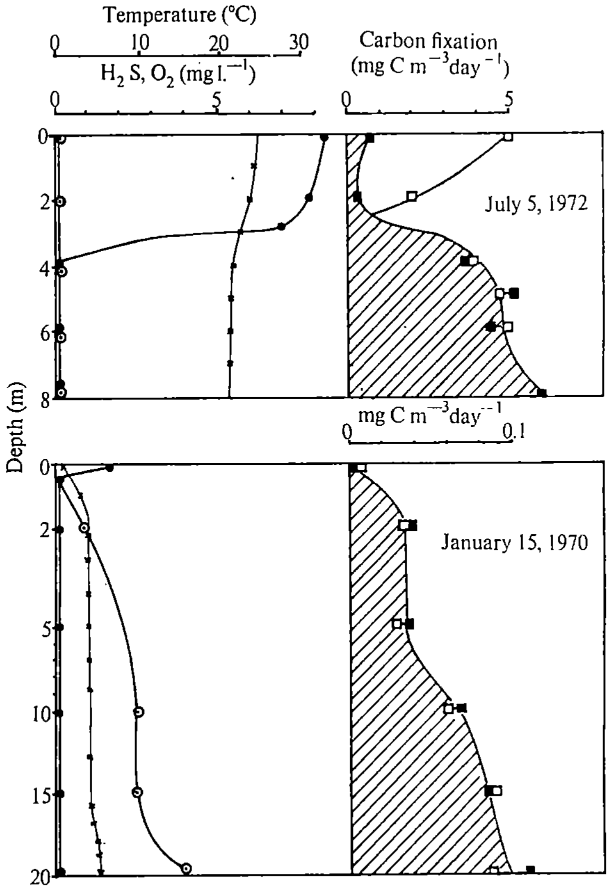

Fig. 1 Vertical distribution of carbon fixation (□, light bottle; ■, dark bottle), oxygen (●), hydrogen sulphide (○), and temperature (×) in Lake Katanuma.

So far as we know, there is no information about the existence of CO_2 assimilating microorganisms in such anoxic and strongly acidic water. Thiobacilli, iron bacteria and algae can survive in acidic water of about *p*H 2, but not in anoxic conditions. On the other hand, *Desulfovibrio* is active in an anoxic environment but only in water above *p*H 4 (refs. 3 and 4).

Our evidence of microbial CO_2 uptake in the dark, in anoxic and strongly acid water provide ssignificant information regarding the limit of existence of microorganisms in the natural environment.

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Granules Containing Lead in Isolated Mitochondria

THE divalent cations of calcium, strontium, manganese, barium and magnesium can accumulate in mitochondria[1]. In spite of variations in rates and in requirements for chemical components of the incubation medium, uptake of these cations leading to granule formation is dependent on energy[1]. We report here the accumulation of lead in granules produced in isolated rat liver mitochondria following incubation in media containing lead. The computed levels of free lead ion in the media ranged from 0.2 μmol to 100 μmol; by comparison, total plasma lead levels of up to 8 μmol have been found in victims of lead poisoning[2].

Mitochondria were isolated with as little disruption as possible by layering a 0.25 M sucrose homogenate on 0.34 M sucrose and centrifuging at 500*g* for 10 min in a Sorvall RC-2 refrigerated centrifuge. The supernatant was then spun at 2,000*g* for 10 min to recover the mitochondrial pellet which was used in these experiments. As controls, mitochondria were incubated at 37° C for 30 min in the following calcium-free medium: 80 mmol NaCl, 10 mmol $MgCl_2$, 10 mmol succinate and 5 mmol ATP adjusted to *p*H 7.2 with 10 mmol Tris-HCl and 4 mmol phosphate buffers. The experimental samples were incubated in this medium with the addition of 1 mmol lead nitrate and 1.5 mM nitrilotriacetic acid (NTA). The granules formed under these conditions are illustrated in Fig. 1*a*. (The concentrations of lead and NTA were chosen so as to avoid precipitation of lead chloride and lead phosphate in the medium.) Using published *p*K and stability constant data for lead ion with the ligand NTA[3], the amount of free lead ion in the solution was calculated to be 2×10^{-4} mM at *p*H 7.2. If the phosphate was omitted from the medium and the NaCl replaced with an equiosmolar concentration of sucrose, the level of free lead ion could be raised to a computed level of 0.1 mM; this was also possible using 0.2 mM lead nitrate and 0.3 mM aspartic acid instead of the previous lead–NTA mixture. Under these conditions significantly larger lead granules were formed (Fig. 1*b*).

For electron microscopy, samples were cooled to 4° C, spun at 2,000*g* for 5 min, washed with 0.25 M sucrose and centrifuged for a further 5 min at 2,000*g*. The pellets were resuspended, and fixed, in veronal acetate-buffered 1% OsO_4 for 0.5 h, dehydrated in acetone and embedded in 'Durcupan'. After sectioning on a 'Reichert' ultramicrotome the specimens were examined using a Philips EM 200 electron microscope.

The granules that were formed when the mitochondria were incubated in the media containing lead were extremely dense and could be seen in the matrix around the cristae and other regions of the inner membrane. Incubation of mitochondria in the presence of 4 mM $CaCl_2$ led to the formation of granules that were less electron-dense (Fig. 1*c*), whereas if lead and calcium were both absent no granule formation was observed. Other investigators[1,4,5] have shown that calcium ions are actively accumulated by the mitochondria incubated under similar conditions and that the resulting electron-dense granules have a tertiary calcium phosphate composition. Other calcium phosphate-containing mitochondrial granules have been observed in many cell types—including chondrocytes—under normal physiological conditions[6,7]. It has been suggested that the potential for calcium uptake by mitochondria is ubiquitous in the tissues of higher animals[7].

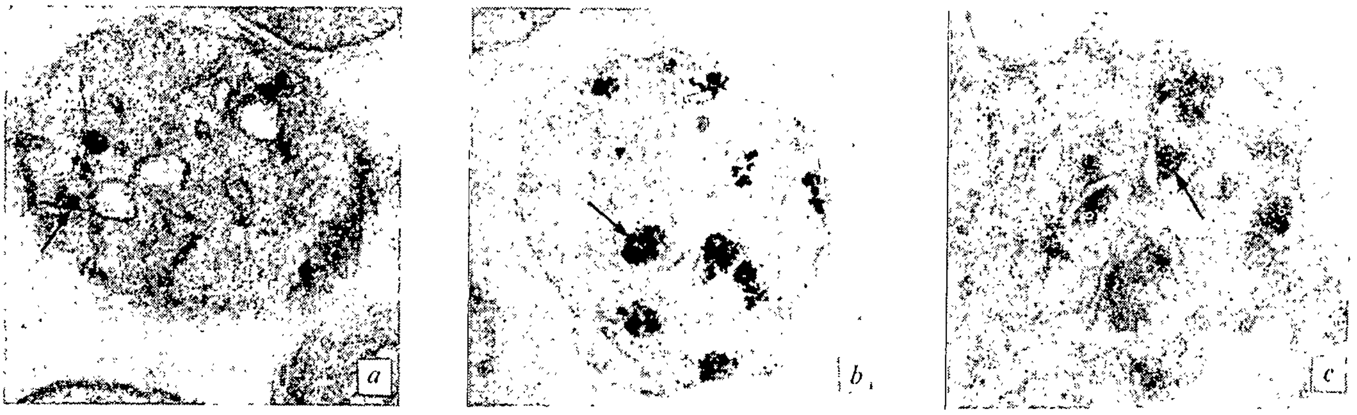

Fig. 1 Electron-dense granules (arrows) in isolated rat liver mitochondria. Osmium-fixed, unstained. *a*, Granules containing lead produced with NTA-metal buffer. ×62,000. *b*, Granules containing lead produced with aspartic acid-metal buffer. ×62,000. *c*, Granules containing calcium. ×69,500.

When succinate and ATP were omitted from the incubation media containing lead, lead was not observed within the mitochondria. Addition of antimycin A, dinitrophenol (DNP), or sodium azide to the media at the beginning of the incubation period resulted in significantly fewer and smaller dense granules. These substances are metabolic inhibitors which operate at different points in energy pathways. Ouabain, which is an inhibitor of some K^+-dependent ATPases but which has little effect on mitochondrial breakdown of ATP, was inactive. These results imply that the mitochondrial uptake of lead is a process dependent on energy.

A difference between calcium and lead-rich granules was observed in further incubation for 60 min in the basic medium containing 0.5 mM DNP. Whereas calcium granules became largely dispersed[5], the lead granules seemed to be unaffected, indicating that lead removal is a less readily reversible process. This is consistent with the observation that lead binds much more strongly than calcium to some ligands (for example, sulphydryl and phosphate groups[3]). It would be expected therefore that if mitochondria accumulate lead in clinical lead poisoning as they do calcium under normal conditions, the subsequent removal of lead from the mitochondria would be physiologically difficult.

It is suggested that the effects of lead on the mitochondria are two-fold. First, by analogy with calcium uptake[1], diversion of energy derived from the electron-transport chain to the active accumulation of lead would be expected to prevent ATP synthesis and to interfere with the maintenance of ionic concentration gradients across the membrane barriers[8]. Second, lead in the mitochondria would be expected to hydrolyse ATP[9], form complexes with the SH^- groups of mitochondrial enzymes, such as those involved in cytochrome synthesis, and to react with other matrical anions including phosphates. The terminal result of excessive accumulation of lead cations by an intact cell would thus lead to a disruption of the ability of the cell to maintain itself metabolically and structurally. This failure would result in death and dissolution of the cell. These processes may account for the fibrosis observed in the liver and kidney of lead-poisoned victims.

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GENERAL

New Problem in Concept Formation

THERE is a simple rule that defines which of the symbols of Fig. 1 fall into group A and which into group B. It can be expressed in thirteen words, but the reader is unlikely to guess it—even if we tell him that the only relevant differences between the patterns are in symmetry, dot colour and dot shape.

The rule is that an A symbol is at least two of symmetric, black and composed of circles. The difficulty is that no single property is necessary or sufficient to make a symbol a member of either group. We therefore call this a polymorphous rule, following Ryle[1] and Urmson[2]. We can define an *m*-out-of-*n* polymorphous rule as one in which there are *n* relevant conditions of which *m* must be satisfied; Fig. 1 involves a two-out-of-three polymorphous.

We have done several experiments to prove that polymorphous rules are exceptionally difficult to find. We report two experiments here: they compare the polymorphous rule with the disjunctive and conjunctive rules studies by Bruner, Goodnow and Austin[3]. A conjunctive rule (for example "an A is both black and symmetric") has necessary but no sufficient conditions; it could be called an *n*-out-of-*n* polymorphous. A disjunctive rule (for example "an A is either black or symmetric") has sufficient but no necessary conditions; it is a one-out-of-*n* polymorphous.

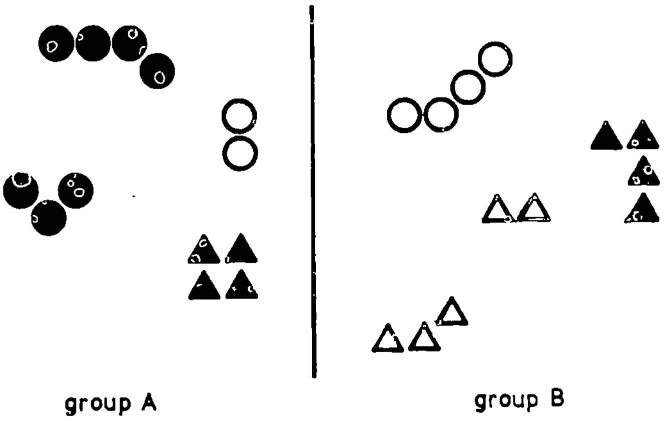

Fig. 1 Patterns determined by a two-out-of-three polymorphous rule.

In experiment 1, seven undergraduates each tried to solve three problems. In each, they were given a pack of forty-eight cards and told to expose them one by one. They were told that the pattern on each card belonged to one of two groups, A and B, and that they must try to find and use the rule that determined which patterns belonged to group A. As the subject exposed each card, he guessed whether it belonged to group A, and was told "yes" or "no". If he made eight consecutive correct guesses he was asked to state the rule as best he could. Any rule was accepted if it correctly assigned all forty-eight cards without simply enumerating them; giving an acceptable rule ended the test.

Table 1 Dimensions of Variation in the Three Packs of Stimulus Cards

	Pack 1	Pack 2	Pack 3
Nature of material	Arrays of typewritten letters	Rows of shapes	Random shapes
Dimensions	Colour (black/red)	Number in row (odd/even)	Outline (smooth/jagged)
	Symmetry of array (symmetric/not)	Shape (square/circle)	Colour (blue/green)
	Letter (x/o)	Shading (filled/empty)	Shading (hatched/empty)

Each subject faced one conjunctive, one disjunctive and one two-out-of-three polymorphous problem. In each there were three two-valued dimensions of variation in the patterns, but in the conjunctive and disjunctive problems one of these was irrelevant. The experimenter told the subject what the three dimensions were. Three packs of cards, using different sets of dimensions were used; the symbols are summarized in Table 1. All six possible orders of presentation of the three problem types were used, and the assignment of card pack to problem type was balanced between subjects. For all problems, there were twenty-four group A cards in the pack.

All seven subjects performed worst on the polymorphous problem. The median numbers of cards exposed before the last incorrect guess were nine, twenty-eight and forty for conjunctive, disjunctive and polymorphous problems respectively. Four subjects did not reach the criterion on solution on the polymorphous problem before the pack was exhausted; those who did solve it gave the rule as a disjunction of conjunctions (for example "an A is black and composed of circles, or symmetric and composed of circles, or black and symmetric") rather than in the simpler "two-out-of-three" style.

The concordance between subjects as to the order of difficulty of the problem types was 0.8 (Kendall's W coefficient of ranks[4]), significant at the 0.001 level. There was no effect of order or pack, and other measures of difficulty lead to similar concordances. Experiment 1 thus showed that disjunctive problems are harder than conjunctive ones (confirming a result of Bruner *et al.*), and that polymorphous problems are harder than either.

In experiment 1, subjects could only acquire information gradually. In experiment 2, all possible information was available from the start. Six more undergraduates served. Cards were selected from the packs used in experiment 1 to make packs of eight including every combination of the three stimulus dimensions. These were laid out on a table, segregated into groups labelled A and B. The experimenter told the subject that a simple rule determined whether a card belonged to group A, asked him to find it as quickly as possible, and measured the time until he offered an acceptable rule. Unacceptable rules were refuted by pointing to counter-examples. The maximum time allowed was 10 min. Each subject faced one problem of each type (conjunctive, disjunctive and two-out-of-three polymorphous); order and pack were again counter-balanced, and the subjects were told before they started each problem what three dimensions might be relevant to it. Their task was thus like the puzzle we set the reader at the beginning of this communication.

Once again, every subject did worst on the polymorphous problem. The median times to solution (scoring 10 min for complete failure) were 34 s, 2 min 35 s and 10 min for conjunctive, disjunctive and polymorphous problems respectively; four subjects failed to solve the polymorphous problem (the other two gave the rule as a disjunction of conjunctions). Kendall concordance between subjects as to order of difficulty was 0.63, significant at the 0.05 level[4]. There was no effect of pack or order of presentation. The two experiments lead to the same conclusion—that polymorphous rules are very hard to discover. The total failures to solve the polymorphous problems prevent us setting figures on their difficulty, but the direction of the result is unambiguous.

Linguistic philosophy gives peculiar importance to the polymorphous rule. Ryle[1,5] suggests that many philosophically important concepts (such as "thinking") and everyday terms (such as "solicitor") have polymorphous definitions, whereas Wittgenstein[6] seems to suggest that such definitions are the rule rather than the exception. Also Neisser[7] has remarked that written letters and spoken syllables, the patterns we recognize most easily and commonly, fall into "ill-defined categories". It is notable that all these polymorphous concepts are generated by human behaviour; and in experiments on spontaneous classification, we have found that subjects use polymorphous rules quite freely. It seems extraordinary that such rules should be so hard to formulate. Yet there is no reason to think the difficulty is not general. We report here experiments using two different test procedures and three different sets of stimulus material; informal tests under the most varied conditions have always produced the same result. Nor is the difficulty entirely in the formulation of the rule: the trials-to-last-error measure used in experiment 1 does not involve explicit formulation. At the least, these experiments point to a difference between rules which are easy to use and those which are easily learned; it may be that this reflects a difference between rules which we generate spontaneously and those which we can extract easily from external problems. This would be evidence against the widely held theory of "analysis by synthesis" (see Neisser[7], page 161ff), which implies that rule discovery inevitably involves internal generation of a corresponding rule.

We thank Dr Hall for discussion.

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Comment

THE preceding communication presents two experiments which investigate the ability of undergraduates to deduce and formulate a classification rule for three problem types. The performance of the subjects is described in terms of two quantitative measures—median number of cards exposed in experiment 1 and median time to solution in experiment 2. As the authors admit, however, the actual numerical values presented are rather misleading because subjects fail to achieve a solution. I cannot understand why the authors did not present detailed qualitative descriptions of the kinds of rules offered by the subjects in each task.

The chief conclusion of the communication is that in the experimental conditions used by Lea *et al.*, *m*-out-of-*n* polymorphous problems are very difficult to formulate. The authors remark, however, that some workers have claimed that polymorphous rules are those most frequently used in normal cognitive activity and they mention that they have obtained experimental evidence (in conditions which are not clearly specified) which suggest that subjects can and indeed do choose to use polymorphous rules frequently. It seems to me that the authors should ask themselves why, with their experimental procedure, instructions, and stimulus material, the subjects failed to show the supposed fluency with polymorphous problems. It is worth noting that several workers have remarked that we habitually categorize and assign structure to events using rules which we cannot formally define—see work on visual and auditory pattern recognition and interpretation, and linguistics.

Lea *et al.* argue that because they obtained their results using two "different" test procedures (by in fact varying the number of visible stimuli against which the subject could evaluate a rule), three different sets of stimuli (by varying the selection of relevant stimulus dimensions but not by sampling different classes of stimuli) and in informal tests under the "most varied conditions" (unspecified) there is no reason to doubt the generality of the finding. This statement may or may not be correct but the authors give little information with which to evaluate their claim. The authors finally suggest that the apparent conflict between their results and the supposed generality of polymorphous concepts may reflect the fact that polymorphous concepts are hard to learn but, once learned, are easy to use. The discussion which follows this remark contains a number of implicit assumptions which to my eyes move the discussion into the realms of the inscrutable.

The interest of the authors' claim must depend on how adequately an experimental procedure which involves the presentation of meaningless patterns yields a representative measure of the human capacity to deduce and test a conceptual role.

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BOOK REVIEWS

History of Biochemistry

Molecules and Life: Historical Essays on the Interplay of Chemistry and Biology. By. J. S. Fruton. Pp. x+579. (Wiley-Interscience: New York and London, November 1972.) £8.40.

FRUTON gained general respect because of his early work on peptides which led to the synthesis of substrates for various proteolytic enzymes—including pepsin. A generation of students is indebted to him, and Simmonds, for a text book of biochemistry. Now he has written an excellent history of biochemistry. Even finding out the dates and first names of nearly 1,500 people listed in the index must have been a formidable labour. But the book is not just a catalogue of scientists and their work, it is a closely argued discussion of their philosophical outlook, influence and obsessions. On the whole, Fruton is kind. He concentrates on the discoveries that scientists made rather than on their mistakes and illusions. He says that he has tried to avoid classifications into "good guys" and "bad guys" and apologises for any lapses into the "Whig interpretation of history" which sees developments, not through contemporary eyes, but by hindsight. So as to avoid too much influence of hindsight, the copious quotations he includes, dealing more with research than with general survey, are usually taken from early papers rather than from autobiographies or accounts written long after the research was completed. Fruton mentions several examples of faulty memory, or the wish to present a tidy account, leading scientists, writing late in life, seriously to misstate the actual steps by which they made discoveries.

The book does not consider the whole of biochemistry in a continuous historical narrative. Instead it is divided into six sections. The first deals with the physical environment in which biochemists worked. Fruton traces the development in different countries of research by amateurs, in universities and in research institutes; the development of scientific societies; and the proliferation of journals.

The study of fermentation may be regarded as the beginning of biochemistry: the importance of yeast in our subject is therefore fittingly commemorated in the derivation of the word enzyme. Enzymes are the subject of Fruton's second section. He traces the slow acceptance of the idea that fermentation was one of yeast's ways of life, the establishment of the idea as a dogma, and the demolition of the dogma when Buchner, who was trying to make an antitoxin, got fermentation with a cell-free yeast extract. In Jacques Loeb's phrase, "Biology was relieved of another fragment of mysticism."

Emil Fischer gets most citations in the index, perhaps because Fruton's main personal interest is peptides. Liebig comes second. He dominated the mid-19th century, both because of his sound work on pure chemistry and on enzymes, and because of his forcefully expressed but often erroneous opinions on agriculture and metabolism. He did not escape criticism; thus Berzelius wrote: "This easy kind of physiological chemistry is created at the writing desk, and is the more dangerous, the more genius goes into its execution . . ."; Lawes Gilbert demonstrated the falsity of his views on fertilizers and on protein as the source of muscular energy. It is nevertheless clear from Fruton's account that Liebig's influence was, on the whole, beneficial. This leads to the depressing conclusion that there is validity in Francis Bacon's aphorism, "Truth often comes more easily from error than from confusion."

Those who yearn for scientific probity will get more comfort from the third section—on proteins. Eminent scientists are shown persistently to have enmeshed themselves in false assumptions without deluding the mass of their contemporaries. Willstatter thought that enzyme activity was caused by a little something added to a carrier, Bergmann thought that the sequence of amino-acids in a protein must be regular, Langmuir thought that the final configuration must have symmetry and regularity. Other physicists are quoted who, having donned a biological strait-waistcoat of their own invention, grumble because it is uncomfortable. One of the few criticisms I have of this book is that Fruton, when discussing the crystallization of enzymes, does not comment on the curious absence of tests for activities, other than that sought, in the early crystalline preparations. Some carried so many enzyme activities that it was simply preconception that gave them one name rather than another.

The effects of erroneous assumption and premature generalization are important features of the section on nucleic acids. As with proteins, there is the intrusion of the idea that they must be simple and regular. Liebig was exceptional in thinking that proteins from different organisms were often identical, but many biochemists assumed that there were just two nucleic acids, and that all plants contained one and all animals the other. Fruton points out that towards the end of the 19th century nucleic acids were assigned extensive roles, it was false assumption that led to their neglect during the first half of this century.

The fifth section traces the slow development of clear ideas about biological oxidations. Progress here seems to have been inhibited much more by the intrinsic difficulty of doing relevant experiments with biological material than by false assumption. Even so, it is interesting that as late as 1914, W. B. Hardy felt constrained to write, "It must always be remembered that an oxidation place is also a reduction place, and it is to be called the one or the other according to the particular zero which is chosen." Considering the part that spectroscopic measurements now play in the study of enzyme actions and in the identification of substances, it is salutary to remember that MacMunn stressed in 1886 the usefulness of coloured substances in studying the internal processes of tissues: the technique was not extensively used till after Keilin's publications from 1925 onwards.

Some aspects of metabolism and of the internal organization of the cell are the themes of the last section. At one time hypothetical entities, of which protoplasm was the most respectable, were invoked to "explain" everything. Then Hopkins led the revolt, pointed out that these entities were merely words and explained nothing, and called for comprehensible reactions between small molecules. Hopkins may have overreacted against mere size in molecules—but he got rid of a great deal of notional biochemistry and of the idea that biology involved peculiar processes that could not ultimately form part of sciences such as chemistry and physics. Among the many quotations in this excellent book is one from Chevreul in 1824, "Are the nature and effects of physical and chemical forces sufficiently well known at present to allow us reasonably to affirm that a particular phenomenon of living bodies is absolutely independent of them? Certainly not." That is still the basic postulate of biochemistry.

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Roots of Astronomy

Education in and History of Modern Astronomy. Edited by R. Berendzen. Pp. 275. (New York Academy of Sciences: New York, August 1972.) $24.

AT the end of the eighteenth century, an observer of the educational scene in Britain remarked, "It has long been a matter of surprise to those who are interested in the education of youth, that . . . so few attempts have been made to facilitate the study of astronomy". The appearance of the proceedings of this first international conference on education and history of modern astronomy allows us to ask the question—how do things stand now?

Although the present volume does report briefly on astronomical education in the United Kingdom (and also contains statements of the position in Australia, Canada and India), the main emphasis is, hardly surprisingly, mainly on astronomical education in the United States. One gains the overall impression that, though the amount of astronomy teaching at all levels in the USA may not be very large, much more effort is being expended on its development than in the UK. Thus the American work on astronomical films, CCTV, computer graphics, case-study projects and the educational use of planetaria finds as yet only few parallels in Britain. On the other hand, postgraduate astronomical education in the United States seems to face more problems than ours does, since both the relative expansion in postgraduate numbers has been greater there, and the cutback in funding has proved relatively more severe. Astronomy students in both countries might, perhaps, envy recruits to traditional astronomy courses in India who, we are told, can usually find employment "as teachers or start their own businesses as astrologers and/or priests". (On the debit side, these Indian courses have the disadvantage of being taught only in Sanskrit.)

One of the most important emphases in US astronomical teaching lies in the attempt to involve students who have no intention of specializing in astronomy: indeed, who have no wish even to specialize in science. A good deal of imagination is evident in the handling of such students. Thus the University of Southern California runs a course entitled "Astronomy's Contribution to Western Culture". The various projects thought up by students include one—described as "most stimulating"—consisting of a study "that one of our teetotaling students performed on the effect of brandy on limiting magnitude". There is clearly a fairly widespread belief amongst US astronomers that introductory courses of this type, apart from their intrinsic worth, help counter the growing anti-scientific trend amongst American students, not least as regards the revival of astrology. (One of the reported discussions at this conference provided, incidentally, a fine definition—culled from a woman's magazine—of the difference between astronomy and astrology. "Astronomy is when you go outside and look at the sky, and all you see are the stars. Astrology is when you go outside and you look at the sky, and you see dragons and virgins and other creepy things.")

It is evident from the papers and discussions at this conference that putting on broadly-based, culturally relevant courses actually entails more work than putting on specialized astronomy courses. One main reason is that an astronomer's research interests are not normally relevant to the former type of course. Nevertheless, many of the American participants obviously thought the effort worthwhile. What then, in comparison, of the British scene? It seems to me that we fall down just at this point where most emphasis is being brought to bear in the United States. Our education of would-be specialist astronomers is reasonably good, but our provision of astronomy as a non-specialist subject, and at levels below the university, still leaves a great deal to be desired. Surely, interest in the physical sciences is not so vast that we can afford to ignore astronomy, currently one of the most fascinating sciences for the layman? The effort involved in a really major increase of astronomical teaching at an introductory level would certainly be large, but it might prove to be eminently worth making.

A. J. MEADOWS

Electrodics

Electrochemistry. Edited by J. O'M. Bockris. Consultant Editor A. D. Buckingham. Pp. 332. (MTP International Review of Science.) (Butterworth: London, University Park: Baltimore, 1973.) £10; $24.50.

A NOTE by the publishers of the *MTP International Review of Science* states that the aim is to provide regular authoritative reviews of entire disciplines. This volume is the first on electrochemistry and it is devoted almost exclusively to what is sometimes termed electrodics: the study of phenomena and reactions at electrode surfaces. Within this narrow field, which very much mirrors the interests of the editor of the present volume, some useful contributions have been brought together. The first chapter deals with the fundamental theoretical problem of electrodics—the mechanism of the transfer of charge between ions and an electrode surface—and it traces the development of ideas since the first introduction of a quantum mechanical model by Gurney in 1931. This is very much a chapter for specialists but it is useful to have ideas which are otherwise scattered through the literature brought together in a systematic survey. A disappointment is the cursory dismissal of modifications of earlier ideas which the discovery of the hydrated electron may well bring by the *ex cathedra* statement of the authors that in their view "the solvated electron model is to be rejected for normal electrode processes". In the second chapter Professor Conway considers the mechanism of electrode reactions in a more experimental context and particularly where adsorbed species play an important role; this too is useful, but it is inevitably an up-dated and condensed version of his well-known book on Electrode Processes. The survey then changes direction towards metallurgy, and the effect of electrochemical factors on the strength and stability of metals and the participation of metal surfaces in dissolution and deposition processes are considered. An interesting account of hydrogen in metals follows, and this is succeeded by a very detailed survey of the investigation of metal surfaces, particularly at electrodes, by ellipsometric optics. This is highly mathematical, and not easy reading, but it provides a mass of useful material. The final chapter, with an abrupt change of theme, takes an all-round look at electrochemical participation in biological processes; it gives a good insight into what may well prove to be a fruitful electrochemical field.

To sum up, what the survey does is good and it provides rich food for specialists on a miscellany of topics mainly related to electrochemical processes at surfaces, but it is certainly not a systematic survey of the present state of electrochemistry as a whole, and a reader who refers to it with this hope will be disappointed. Printing and illustration are excellent, but there is no index.

A. HICKLING

Environment of Leicester

Leicester and its Region. Edited by N. Pye. Pp. xxi+603+21 plates. (Leicester University: Leicester, September 1972.) (Published for the Local Committee of the British Association.) £6.

FOR many years the annual meetings of the British Association for the Advancement of Science have been marked by the publication of a special volume dealing in a scholarly way with many facets of the host city and its surrounding region. This has usually been promoted by a special local committee in which "town" and "gown" have collaborated in an exemplary fashion, and the general editor has usually been the University's Professor of Geography. The Leicester meeting of 1972 continued these traditions in a most impressive way by the publication of a superb volume containing 600 pages, with ninety-three figures, forty-eight tables and over twenty plates, which, in both size and scholarship, aptly matches the large volume on Nottingham and its region produced for the annual meeting of the Association in 1966. Thus, after a 6-year wait, Leicester has drawn even with its traditional East Midland rival.

It is difficult, of course, if not impossible, to review a work of such length and variety in a conventional way. One can only say that Professor Pye, the editor, and his thirty-five contributors, drawn predominantly from the academic community, but also including representatives of local government and other walks of life, have fulfilled their task with great credit. Each chapter is an up-to-date, authoritative statement on a particular aspect of the city and county that in most cases draws skilfully on the published literature while at the same time offering an additional contribution to our knowledge, whether through fact or commentary.

The book falls into several main sections. After an introduction by Professor Pye, part 1 deals with the physical background (geology; geomorphology; weather and climate; soils; flora and vegetation; fauna; and nature conservation), and part 2 traces the area's historical development (prehistoric and Roman settlement; Saxon and Danish Leicestershire; Leicestershire 1100–1800; the evolution of Leicester 1066–1835; and Leicester and Leicestershire 1835–1971). Part 3 is devoted to economic structure (communications and transport; agriculture; extractive industries; foundation of the modern industrial pattern; the modern industrial scene; science, technology and local industries; patterns of population change; and urban structure of Leicester). Finally, part 4 deals with social development (schools in Leicester; education in Leicestershire; further and higher education; the development of medicine and social services; the arts; vernacular architecture; and the planning of the Leicester of the future).

While the volume is intended to offer a study of a particular English industrial city and its hinterland, the specific experience and characteristics of Leicester and Leicestershire nevertheless almost constitute a "model" case. Here in the very heart of England we have a county still firmly dominated by its ancient capital, which is surrounded by a number of subordinate urban centres with their own local spheres of influence —the epitome of a geometrically-arranged hierarchy of "central places". The physical environment includes a representative sample from the geological time scale, including both the Pre-Cambrian and the Pleistocene. There is a fairly wide range of mineral resources, including coal, ironstone, roadstone and brick earths. Leicester itself exhibits tangible evidence of all the major phases of English urban development, including the remains of the Roman town of *Ratae Coritanorum*, mediaeval churches and Guildhall, Georgian town houses, Victorian working-class factory districts, and the suburban sprawl of the present century. (It is scarcely surprising that the local College of Education has sponsored the idea of "town trails" in Leicester.) Modern Leicester is large enough to exhibit the well-differentiated commercial, industrial and residential neighbourhoods so typical of Western cities (and thus incidentally lends itself to factor analysis and other techniques of the modern urban analyst). The city's industrial evolution shows the typical change from a varied structure of numerous small handicrafts and trades to reliance on one or two dominant manufacturing industries, in this case hosiery and footwear, that began as domestic activities and became converted to a steam-powered factory basis after the middle of the nineteenth century. The twentieth century has dealt kindly with Leicester, so that, unlike the experience of some northern industrial towns, the spontaneous growth of new industries, dominated by engineering, has diversified a potentially vulnerable industrial structure and taken up the slack caused by the falling or static labour requirements of hosiery and footwear.

While there is so much that is typical of the country as a whole, there are, however, important achievements and innovations to Leicestershire's own credit. For example, modern livestock breeding was pioneered by Robert Bakewell. The hiring of a special train from Leicester to Loughborough by Thomas Cook marked the advent of the modern tourist movement. Frank Whittle nurtured his jet engine in local factories during the late 1930s and early 1940s. And, quite understandably, important developments took place here in the technology of hosiery and footwear manufacture. More recently, the "Leicestershire Plan" for education, leading to the establishment of separate upper schools for all pupils over 14, and the "Leicester Traffic Plan", involving the segregation of pedestrians and vehicles and the shielding of the city centre from the motor car, have both achieved fame for their bold imagination.

Leicester and its Region will be used chiefly as a valuable reference book for the upper forms of schools and for institutions of higher education, especially where geography, local history and environmental studies are concerned. In that role it will, over many years to come, handsomely vindicate the labours of all those who have contributed to it.

R. H. OSBORNE

Catecholamines

Handbook of Experimental Pharmacology/Handbuch der Experimentellen Pharmakologie. Edited by H. Blaschko and E. Muscholl. Pp. 1,054. (Springer: New York and Berlin, 1972.) DM396; $125.60.

THIS tome on the catecholamines is volume 33 of the new version of the famous Heffter's *Handbook of Experimental Pharmacology.* The volume has 1,054 pages and its weight makes "Handbook" a curious misnomer. But the weight is justified by the contents. The enormous increase in catecholamine research represented here is due not only to the universality of their distribution and effects but also because the analysis of their mode of action led to the discovery of the cyclic AMP system which is now recognized as a common mediator of the actions of many other hormones as well. Appropriately, therefore, a masterly account of the effects of catecholamines on metabolism is given in a central chapter by Jean Himms-Hagen, much of which summarizes present knowledge on the effects of catecholamines on adenyl cyclases and on enzymic effects of cyclic AMP.

All chapters are of high quality in both content and style, as would be expected from the well-selected experts who wrote them. It would be invidious to select any particular chapter for praise, but to someone working on amine storage and release mechanisms, the two chapters devoted to these could hardly be improved on.

This book should be available to everyone engaged on any aspect of catecholamine research. The pages are packed with essential information which is remarkably up to date for such a compendium; apparently most contributors were able to include consideration of publications in the first half of 1970. Another attractive feature, traditional for this handbook, is the freedom with which the contributors lay bare their ideas on mechanisms and provide stimulating but concise expressions of opinions about open problems. Their involvement in current research makes these opinions very much worth having. The editors, H. Blaschko and E. Muscholl, deserve congratulations and gratitude from all those interested in the catecholamines. The publishers deserve congratulations for the excellence of production of the book which includes a detailed, accurate index.

G. V. R. Born

Separation Techniques

Pharmaceutical Applications of Thin-layer and Paper Chromatography. Edited by Karel Macek. Pp. xvi+743. (Elsevier: Amsterdam, London and New York, 1972.) Dfl. 250 ; $73.50.

Chromatography is the name given to a series of closely related but technically different separation techniques whereby minute quantities of mixtures can be resolved into their various components. The simplest and cheapest techniques—and thus the most popular—are paper and thin-layer chromatography. One consequence of the popularity has been the extraordinary number of publications dealing with one aspect or another of the technique or of application of the technique in the solution of different analytical problems. It is hard to find a branch of science that has not benefitted from chromatography, although it could easily be argued that the pharmaceutical sciences have benefitted most. A variety of chemical substances, synthetic and natural, will be found in the pharmaceutical literature: psychotropic drugs, antihistamines, antipyretics, analgesics, antitussives, sedatives, hypnotics, anaesthetics, antiepileptics, local anaesthetics, adrenergic and cholinergic substances, diuretics and cytostatics to name but a few of the synthetic substances; alkaloids, glycosides, antibiotics, laxatives, vitamins, to name but a few of the natural products as well as auxillary substances such as antioxidants, colouring matters and preservatives. The analyst may have to examine any one of these drugs for impurities or determine the amount of one in the presence of others. Thin-layer and paper chromatography has almost certainly been used by someone and reported in the literature. The editor, who is bibliography editor of the *Journal of Chromatography*, in association with expert workers with the different groups of substances has compiled a very comprehensive review of more than 2,500 references. Each chapter begins with generalized information about extraction procedures for pharmaceutical dosage forms or from crude drugs and includes useful comments and explanations in connexion with the methods described. The free use of tables summarizes important information such as solvent systems, spray reagents and hRf values.

Prior to this main section of the book there is a short section on techniques—qualitative and quantitative, which, without resource to repetition of much information already detailed in other books, gives facts and advice that are equally useful to the expert and the beginner. The text flows remarkably well and it is only the heading of chapter 9—"The Tasks of Paper and Thin-layer Chromatography" that betrays its Czechoslovakian origin. It is an extremely good translation and those concerned are to be congratulated. The book is well bound, the text is clear and printed on good quality paper with few typographical errors. While it is doubtful whether many British analysts can afford to purchase it, there is no doubt that the book should be in every laboratory concerned with pharmaceutical analysis.

E. J. Shellard

Stress on Plants

Responses of Plants to Environmental Stresses. By J. Levitt. Pp. xii+697. (Academic: New York and London, March 1972.) $32.50.

Jacob Levitt is one of the leading scientists in the field of stress physiology of plants. As a sequel to his comprehensive studies on temperature and drought resistance of plants, Levitt presents a fifth far more detailed and thematically enlarged monograph, which covers most of the recent literature from all parts of the world up to 1969–70. The references run to nearly 100 pages.

It was not, however, the author's intention to write a compendium complete in every respect, but to give an account of the research according to his own particular point of view. This holds particularly for the sections concerning temperature and water stress, which takes up four fifths of the book. The remaining chapters deal with the influences of the different kinds of radiation, salts, and mechanical factors as well as stresses caused by human civilization.

In an introductory chapter Levitt points out ways to a unified stress concept. To conform with interdisciplinary usage he has used a uniform terminology as, for example, the terms "tolerance" instead of "hardiness" or "freezing" instead of "frost". Levitt contrives to formulate the term "stress" which has its exact definition in physics, which is otherwise used in a less precise way. He makes a clear distinction between "stress" as an ecological agent and "strain" which implies the impact and the effect on the plant.

There are strains of an elastic nature, which occur repeatedly without, however, exhausting the plant, so that the effect is reversible. Or they may be of plastic nature, stimulating the plants into adaptation to the stress. Levitt deals mainly with the plastic strain. Resistance of plants can be brought about by avoidance or by tolerance of a strain.

This concept is applied to the different kinds of stresses set out in analogous schemes. In a concluding chapter Levitt discusses the problem of comparability of the strains of the different stresses. Desiccation, for example, can be caused by single or a complex of quite different stresses such as low temperature, retarded water conductivity, osmotic effects, heat, and so on. Levitt distinguishes between comparative primary, secondary and tertiary stresses. The capability of plants to avoid strains can be ascribed to different causes. But as to how plants tolerate the different strains certain correlations can be stated. Most of the stresses described above claim the tolerance of the plants in a similar way and will finally lead to the same injuries. Thus a uniform scheme (system) of injury could be considered possible. In this context Levitt offers his not entirely undisputed theory, developed in 1962, that those injuries are results of oxidative sulphur, sulphur bindings in particular at lipids and proteins—this is a new aspect—at the membranes, which lose their semipermeability. As discussed extensively in the chapter "Molecular basis of freezing injury and tolerance" this concept turns out to be consistent with the hardening of many plant species, which keep a high reducing capacity (that is, many sulphydryl-groups). Levitt endeavours to integrate into his concept many other existing theories, or to refute them. The seasonal "phenological" view of hardening is relatively neglected in favour of the biochemical aspects.

This excellently written book will be an indispensable source of knowledge and information for physiologists, ecologists and all students of applied biology. It should also be considered as an impulse to further critical discussions about causes of the resistance of plants (and animals) to extreme environmental stresses.

L. Kappen

Obituary

Dr L. S. B. Leakey

Louis Seymour Bazett Leakey, M.A., PhD, DSc, FGS, FBA, died in London on October 1, 1972, while on his way to America to fulfil lecturing engagements. Perhaps best known as a physical anthropologist, palaeontologist and prehistoric archaeologist of distinction, he was also a man of many talents.

Louis Leakey was born in 1903, the elder son of missionary parents who were working among the Kikuyu at Kabete near Nairobi. From his earliest days his schoolmates were from this tribe so that his command of the Kikuyu language was unrivalled by any other European. Taught at first by his parents and a governess, Leakey was prevented by the Great War from attending an English school until he was over 16. In 1922, he entered St John's College, Cambridge, as a Sizar; some 44 years later he was made an Honorary Fellow of the College in recognition of his worldwide achievements. At Cambridge he studied Modern French, Medieval French and Kikuyu for Part I of the Tripos, an unheard of combination. He went on to take a first and then another in Archaeology and Anthropology. As a result of his academic performance at Cambridge and his experience in Africa he was awarded a research studentship that took him on a number of archaeological expeditions. Later, in 1929, he was made a Research Fellow of St John's and took his doctorate.

While at Cambridge he suffered a rugby injury and was advised to recover in the "fresh air". He took a year's leave and accompanied Cutler, a Canadian palaeontologist, who was just leaving for Tanzania to collect fossils for the British Museum (Natural History). This invaluable period in the field, under expert guidance, gave him both practical experience and a basic knowledge of palaeontology. At first Leakey's principal interest was in the sequence of prehistoric cultures in Kenya. He attempted to correlate cultural changes with climatic changes during the African Pleistocene in terms of pluvials and interpluvials corresponding with European glacial alternations. During this fieldwork several important, if controversial, finds were made, in particular the Kanam jaw and the Kanjera skulls. In each case the antiquity of the material was doubted, and in one case even the provenance; this pattern of events is well known to human palaeontologists and to others in science whose discoveries disturb the tranquillity of textbook writers.

In the late 1930s Louis Leakey spent over two years in the study of the history and customs of the Kikuyu tribe. The results of this extensive research were written many years ago and they will be published soon as one of his posthumous papers. After the 1939–45 war, in which he served in the African section of the Special Branch CID, Leakey was appointed full-time Curator of the then Coryndon Museum. He added to the Museum a Centre for Prehistory and Palaeontology by attractinig research grants from a number of sources, notably the Wenner-Gren Foundation for Anthropological Research and the National Geographic Foundation. By now the tide of his discoveries was beginning to flood; sites such as Rusinga, Olorgesailie and Olduvai producing important new finds.

The recovery of the 1948 "*Proconsul africanus*" skull was of great significance as it was the earliest ape skull then known. It was examined by Leakey and Le Gros Clark along with a great deal more material recovered from the Kenyan Miocene. The resulting monograph *The Miocene Hominoidea of East Africa* is still a foundation reference for those embarking upon primate palaeontology. The particular importance of the 1948 "*Proconsul africanus*" skull was that at that time it was considered to be the common ancestor of both modern apes and modern man. Subsequent finds made by Leakey, and work by others now make this unlikely. Leakey remained convinced, however, that the common ancestor of man and the modern apes would be found in Africa. The discovery of the Olorgesailie tool site was a major triumph as it remains one of the most significant collections of palaeolithic stone tools ever found.

Soon after the war Leakey was the driving force behind the first Pan-African congress on Prehistory and Quaternary Studies. The first meeting was held in Nairobi in 1947; it has continued at intervals of four years and has proved to be a valuable link between prehistorians, geologists and anthropologists working in Africa. The name of Leakey will always be associated with Olduvai Gorge not only because of Louis but also because of his wife, Mary, who still works at the site. It was from Olduvai that his major success came, aided as always by his family. In 1959 Mary Leakey found the skull of "*Zinjanthropus*" (*Australopithecus boisei*) in Bed I. The skull is a magnificent example of the larger form of australopithecine; the first of its kind from East Africa and reliably dated at 1.75 m.yr. BP. Numerous other finds came from Olduvai including the remains of the smaller *Homo habilis* and from a higher level an example of *Homo erectus*, the maker of an early handaxe culture at Olduvai. At Fort Ternan excavations in Late Miocene deposits produced the maxilla of "*Kenyapithecus wickeri*": still one of the earliest known hominid fragments but now usually accepted as *Ramapithecus wickeri*.

In scientific circles Leakey was always a controversial figure in a field renowned for controversy; he was an eager contestant in verbal or written debate and a keen participant at international meetings. He was the author of over twenty books and 120 scientific papers. Recognition came to him from the worldwide scientific community; he was awarded the Swedish Andre medal, the Cuthbert Peek medal of the Royal Geographic Society, the Viking medal of the Wenner-Gren Foundation for Anthropological Research, the Pitt-Rivers memorial medal of the Royal Anthropological Institute and, with his wife, Mary, the Prestwich medal of the Geological Society of London (the first time that this medal has been jointly awarded to a husband and wife team).

Only the perceptive of history will allow a true evaluation of Leakey's place in science since many of his opinions have yet to be vindicated or disproved. In the meantime we can only admire his enthusiasm, his initiative, his diligence and his consistent success in the search for fossils and stone tools. A man of greatness and courage who fought hard for the new knowledge that he gave to the world; a man who gave unstintingly of himself to the study of palaeontology, prehistory and the evolution of man.

Announcements

Miscellaneous

THE following have been awarded Alfred P. Sloan Research Fellowships. **Arizona State University:** Alexandra Navrotsky. **California Institute of Technology:** Michael Aschbacher, Jeffrey E. Mandula, Robert W. Vaughan, Michael W. Werner. **Stanford University:** John H. Coates, Theodor W. Hansch. **University of California, Berkeley:** Carl E. Heiles, Wayne L. Hubbell. **University of California, Irvine:** Robert T. McIver, Jr. **University of California, Los Angeles:** Thomas M. Liggett, Malcolm F. Nicol, Bruce L. Rothschild. **University of California, San Diego:** Carl H. Fitzgerald. **University of California, Santa Barbara:** David S. Cannell. **University of Colorado:** Arlan D. Norman. **Yale University:** Ronnie Lee, Bernard Shiffman. **Northwestern University, Evanston:** George Gasper, Jr. **Southern Illinois University, Carbondale:** Conrad C. Hinckley. **University of Chicago:** Robert D. Carlitz, Ronald H. Kluger, Paul B. Moore, Anthony D. J. Robertson. **University of Illinois, Urbana:** E. Graham-Evans, Jr., J. Douglas McDonald. **Iowa State University:** Jon C. Clardy. **University of Kentucky:** Thomas A. Chapman. **University of Maryland:** David D. Brayshaw. **Brandeis University:** David Eisenbud. **Harvard Medical School:** John G. Hildebrand. **Harvard University:** Bryan E. Kohler. **Massachusetts Institute of Technology:** Carleton DeTar, Michael S. Feld, Norberto Kerzman, K. Barry Sharpless. **Michigan State University, East Lansing:** Stanley R. Crouch, Richard T. Miller. **University of Michigan, Ann Arbor:** Hans H. Brintzinger. **University of Minnesota:** Ernest Coleman, Donald G. Truhlar. **University of Washington, Missouri:** Alfred G. Hortmann. **Princeton University:** Edward J. Groth, III. **Columbia University:** C. Herbert Clemens, Paul G. Richards. **Cornell University:** Stuart J. Edelstein, Martin F. Semmelhack. **New York University:** Mark A. Ratner. **Rockefeller University:** Anthony Zee. **State University of New York at Stony Brook:** Philip B. Allen, Barry M. McCoy. **University of Rochester:** Stephen L. Olsen. **Duke University:** Thomas T. Norton. **University of North Carolina:** Reuben D. Rieke. **Case Western Reserve University:** Robert C. Dunbar, Raymond J. Lasek. **Reed College, Oregon:** Stephen W. Arch. **University of Pittsburgh:** David Jasnow, Ralph Z. Roskies. **Brown University:** James T. McIlwain, Langley A. Spurlock, Terry E. Tullis. **Vanderbilt University:** William B. Peatman. **Rice University:** W. Edward Billups. **University of Texas:** Kenneth E. Gentle, Jack B. Swift, Robert E. Wyatt. **University of Utah:** John P. Simons, Chin-Hsien Wang. **University of Virginia:** W. Lester S. Andrews. **University of Washington, Seattle:** John M. Wallace. **West Virginia University:** Mohindar S. Seehra. **University of Wisconsin:** Gerald L. Hazelbauer, Max G. Lagally, John L. Schrag. **Queen's University, Kingston, Ontario:** Ian P. Johnstone. **University of British Columbia, Vancouver:** Walter N. Hardy. **University of Waterloo, Ontario:** F. R. McCourt. **University of Windsor, Ontario:** Gordon W. F. Drake.

Errata

IN the article "Stereoselectivity and Differential Metabolism *in vivo* of Dextro and Laevo-Methadone-1-^{3}H" by A. J. Misra and S. J. Mule (*Nature*, **241**, 281; 1973) the following corrections should be made:

Table 1, in the half-life column substitute 1.2 in place of 1.02; paragraph 2, line 18, substitute 55.49±2.15 (s.e.) for 57.3, and 29.77±0.59 (s.e.) for 28.3. Legend for Fig. 3, line 13 "residues" should read "residue"; paragraph 2, line 32 should read " . . . differences in reversible plasma protein binding of two isomers . . . "; paragraph 3, line 4, the reference above "*l*-isomer" should be deleted; paragraph 5, line 8, "*d*-methadone" should read "*d*-methadol"; paragraph 5, line 2, the reference above '*l*-isomer" should be deleted.

IN the review of *Stabilité Structurelle et Morphogénèse*, by René Thom (*Nature*, **242**, 207; 1973), the bibliography omitted to mention that Professor Thom's book is one of a series whose general editor is A. S. Wightman. The location of W. A. Benjamin was given as New York, instead of Reading, Massachusetts, which is the correct location.

International Meetings

June 2–8, **Premiere Semaine Mondiale de l'Innovation** (Inova 73, 62 Rue de Miromesnil, 75008 Paris, France).

June 4–7, **The 24th Annual Meeting of the Tissue Culture Association** (Local Committee Chairman, Dr George Yerganian, Children's Cancer Research Foundation, 35 Binney Street, Boston, Massachusetts 02115).

June 5–6, **The Quality of Life** (The Secretary, Institute of Rural Life at Home and Overseas, 27 Northumberland Road, New Barnet, Hampshire).

June 6–8, **Mode of Antibiotics on Microbial Walls and Membranes** (The Associate Executive Director, The New York Academy of Sciences, 2 East 63rd Street, New York, NY 10021 USA).

June 11–13, **Recent Advances in Staphylococcal Research** (The Associate Executive Director, The New York Academy of Sciences, 2 East 63rd Street, New York, NY 10021, USA).

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NATURE Volume 243 MAY 18 1973

What is *Nature* for?

JOURNALS which too frequently assault their readers with introspection about their functions run several risks, the most immediate of which is that they will be boring and the most serious that they will be so enmeshed by policy statements and declarations of objectives that they find themselves having to choose between flexibility and consistency. In the nature of things, the relationship between a journal and its readers is bound to be—and should be—so much less tangible that formal definition is not merely impossible but undesirable. Journalism, after all, is a means by which a group of readers is given a sense of community by its dependence, however incomplete, on what is published issue after issue. This is the sense in which a journal belongs as much to the readers as the writers. *Nature* has been fortunate, in the past 103 years, in the strength of this sense of community. It is a constant pleasure that readers and contributors regard the journal with fondness—and those who complain do so, more often than not, in sorrow, not anger. The special quality of *Nature* stems as much from this intimacy as from the way in which it has been able to publish the first accounts of some of the distinguished research. And there is no doubt that this special intimacy between readers and contributors will continue even though there is about to be a change of editor (see page 110)—a rare event. Dr David Davies, who takes up this appointment at the end of August, will be only the fifth editor of *Nature* in more than a century (a calculation somewhat complicated by the joint editorship of Mr A. H. V. Gale and Mr L. J. F. Brimble from the Second World War until Mr Gale's retirement and Mr Brimble's later death in 1965).

Why has *Nature* succeeded so well? At the beginning, *Nature* was much more a gossip sheet than a learned journal, as can be told from the extracts from old volumes now published in each issue. It was a means of informing scientists of the late nineteenth century, without other means of communication, of events of general as well as special interest. The departure of the Challenger on its cruises, or the preparations for the next eclipse, were the raw material of *Nature* for the first several years. From the start, however, there was information about the proceedings of foreign academies, a strong and intelligent approach to the published literature of science (including not merely book reviews but reviews of scientific articles appearing in what were then called the scientific serials) and a miscellaneous range of correspondence dealing as much with natural history and natural phenomena as with the kinds of scientific subjects which now constitute a scientific letter. But from the start, *Nature* was a robust critic of the way in which science was managed. The early issues were full of complaints at the British Government's neglect of such good causes as the need to observe eclipses wherever they occur, the need to manage institutions such as the Kew Observatory intelligently and the need for public education in science. While the sense of intimacy which is the first prize of journalism no doubt sprang from the usefulness of the information which it gathered, its public influence depended on the aptness and outspokenness of its opinions. Only towards the end of the nineteenth century was begun the tradition of communicating news of original discovery directly to *Nature*.

It is fair to claim that such success as *Nature* has since enjoyed stems from the pursuit of the old objectives, implicit though they may have been. To be sure, methods have changed with changing circumstances. The days have gone when a decision to publish or decline a scientific article could be determined in the office, by the reputation of the author or the quality of his address. Inevitably, research has become more and more a pursuit for specialists, with the result that it is hard to work to the old ideal that every reader should, if so inclined, be able to read *Nature* from cover to cover. (Even so, it is something of a surprise that fields such as molecular biology have become in the past decade almost inaccessible to all but the specialists.) The increasing competitiveness of research has also created new pitfalls—in reviewing scientific articles which have appeared elsewhere, for example, there is now a much greater risk than in the nineteenth century that an honest researcher will be deeply hurt if it seems that his own work has not been given proper credit. News and general information is easier to collect but ages more quickly. And opinions, however well-considered, are more easily lost than ever among the general noise of public comment. Yet there is no reason to think that the traditions of the past century have become inappropriate nor to fear that, with modification no doubt, they will lightly be abandoned. There is no reason why the second century should not be even more successful—and amusing—than the first.

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100 Years Ago

A *résumé* of our knowledge, strikingly incomplete as it is, on the subject of sneezing, is given by Dr. Seguin in the third number of the new and excellent *Archives of Scientific and Practical Medicine*. The author's attention was drawn to the subject from his observing a fact, previously well known, that sneezing may be frequently stopped by pressing the fingers on the lips or sides of the nose. No new theory is given to explain the physiology of the phenomenon, and it is stated that naturally most of the air expired during a sneeze escapes through the nose, but that custom has brought about the discharge of a part through the mouth. This we cannot agree with, as it is difficult to believe that custom has much influence on so abrupt an act.

From Nature, 8, 76, *May* 22, 1873

OLD WORLD

Geophysicist to be Editor of *Nature*

Dr David Davies, leader of the Seismic Discrimination Group at the Lincoln Laboratory of the Massachusetts Institute of Technology, is to be the next editor of *Nature*.

Dr Davies will take up his new position at the end of August. Mr John Maddox, who has edited the journal since February 1966 is leaving *Nature* today but until Dr Davies takes up his appointment *Nature* will be edited by Dr Alun Jones the present Deputy Editor.

Dr David Davies is 33 years old and was educated at Nottingham High School and Peterhouse, University of Cambridge from where he graduated in physics in 1961. Since then he has been involved in research in geophysics ; first as a graduate student at the Department of Geodesy and Geophysics at Cambridge, then as a senior assistant in research at the same department and, since January 1970, at MIT. Dr Davies was also a fellow of Peterhouse from 1946 to 1969.

Dr Davies's early research was concerned with the exploration of the ocean floor and its underlying geology by seismic methods. During this time he participated in cruises in the Atlantic Ocean, Indian Ocean, Gulf of Aden and the Red Sea. It was as a consequence of this work that Dr Davies developed an interest in providing a theoretical explanation for signals recorded as a result of explosions and his doctorate thesis was also devoted to these problems.

Since 1966 Dr Davies's interests in seismology have broadened and in 1968 he was the rapporteur to the Seismic Study Group convened by the Stockholm International Peace Research Institute.

At this meeting eleven seismologists from ten countries reconsidered the evidence bearing on the detection and identification of underground nuclear weapons tests. One of the results of this meeting is that there is now wide agreement on the scope and limitations of seismology in policing any test ban. The inadequacy of seismic monitoring had been one of the chief reasons why the Test Ban of 1963 had not included underground explosions.

During the past three years Dr Davies has been deeply involved in the remaining problems associated with the policing of a test ban treaty. In particular, he has used arrays of seismic detectors spread over areas of diameter two hundred kilometres in Montana to determine whether nuclear explosions can be distinguished from earthquakes. As well as what Dr Davies calls this "mission oriented research" the arrays have been used to determine the nature of the Earth's core and to determine how seismology can be used in plate tectonics. Recently the existence of lateral heterogeneity deep in the Earth has also been detected by such large arrays.

In 1966 Dr Davies edited a special volume of the *Geophysical Journal* of the Royal Astronomical Society and soon afterwards he became an editor of that journal. He is now one of its two North American editors. Dr Davies has also been *Nature's* geophysics correspondent since 1968.

PHARMACEUTICALS

Drug Research

Large pharmaceutical companies tend to produce more results from their research and development than small ones. This conclusion, in direct contradiction to that reached by the Monopolies Commission, was presented recently at a conference on innovative activity in the pharmaceutical industry organized by the National Economic Development Office. Mr George Teeling-Smith, Director of the Centre for the Study of Industrial Innovation, presented data from a report that is due to be published shortly by the CSII, which shows that there is a linear relationship between the output of new products and research and development expenditure. This contrasts with the non-linear relationship found by the Monopolies Commission.

The difference between the two conclusions is probably explained by the fact that CSII did not use the conventional measure of pharmaceutical companies' research success—the number of patents each company files. Conclusions based on patent filings could be dangerously misleading, Mr Teeling-Smith said, because, for example, companies file patents at different stages in the development of drugs.

Instead of counting patent filings, Mr Alan Angilley, who carried out the work for CSII, attempted to identify all the new pharmaceutical compounds introduced to world markets between 1958 and 1970. Inevitably coverage was incomplete for compounds originating in, for example, Japan, but it clearly showed that the laboratories producing the largest number of innovations also produced those that were the most successful.

Mr Angilley then studied in detail twenty companies who could provide an account of their new products and their research expenditure over a period of one year. The firms chosen represent "a good spread across the spectrum of expenditures in the industry", according to Mr Teeling-Smith, and the study showed that the larger companies tended to be the more productive.

Clearly much larger studies need to be carried out, but CSII's work does suggest that the "conventional wisdom" which led the Monopolies Commission to oppose the Beecham, Boots, Glaxo merger should "be put into cold storage until further soundly-based studies can throw further light on the whole subject", according to Mr Teeling-Smith.

The Monopolies Commission was also criticized by Mr R. D. Douglas, Director and Legal Adviser to Pfizer Ltd, who suggested that because "winners" in the pharmaceutical industry are so elusive and unpredictable, companies rarely enjoy really high profits. Those made by Hoffman-La Roche, which the government is currently criticizing, were calculated by the commission in a "surprisingly crude way", Mr Douglas said. Of greater danger, he said, were the new breed of pharmaceutical companies which marketed at cut prices drugs that others had developed and patented. These companies succeed because actions over patent infringements take so long to complete. One such action, started in 1963, was not concluded until last year.

The time scales involved in drug development are lengthening, and this also worried delegates to the conference. The delay between the initial screening of a compound and its launch is growing, as is the time it takes to reach peak sales.

Equally the time the Medicines Licensing Authority takes to grant permission for clinical trials was criticized by Dr D. G. Davey, Director of Research at ICI, although Dr F. Hartley and Professor O. L. Wade, two members of the commission, suggested that the remedy lies at least partly with the industry. Good submissions can be processed more quickly, they said.

ENVIRONMENT

Charting Chemicals

from a Correspondent

AN information network on chemical substances and their effects on the environment would be valuable, according to the Royal Society's British National Committee for Problems of the Environment. This was the outcome of a meeting held recently in London under the auspices of the committee and attended by 200 participants, which included representatives from the European Economic Commission, the OECD, and the United Nations Environment Programme.

The purpose of the meeting was to discuss the feasibility and usefulness of collecting into a centralized network existing data on known chemicals in order to predict any possible future adverse effect from chemicals with broadly similar properties. In the wider context these data could provide a guide to environmental management policies.

The meeting also called for a reconciliation of the voluntary methods used in Britain for controlling substances introduced into the environment with the legally enforced methods used by most other countries within the EEC.

It was pointed out at the meeting that there was very little known about ecological toxicity compared with the great deal of information available on human toxicology. A call was also made for the participants at the meeting to provide specimen input on relevant environmental sources so that a demonstration project could be mounted in time for the first session of the governing council of the United Nations Environment Programme which is to be held next month.

The conclusions of the meeting, which was organized by Sir James Cook and Sir Frederick Warner, will be discussed by the British National Committee with a view to the committee formulating recommendations which might be put to the government at some time in the future.

SPACE

Belgians Protest

THE European Launcher Development Organization may yet refuse to die quietly. When the French and Germans announced at ELDO's last council meeting on April 27 that they were withdrawing financial support from the Europa II launcher project as of May 1, the chief reaction was silence. Even the British press, which might have been expected to announce that Britain had been right to pull out of the organization in 1968, largely ignored the event. It looked as though the organization could be killed far more easily than many had thought possible.

But Belgium, which only found out about the Franco–German decision in the council chamber, has decided, after thinking about it for a fortnight, to register its protest, and the staff of ELDO has issued a statement explaining exactly what it thinks of the organization's history.

In a statement issued last week, Mr Charles Hanin, the Belgian Science Policy Minister, and chairman of the European Space Conference announced that he intended to convene a meeting of the conference shortly, and made it clear that he is far from happy with the attitude of the French and German governments. Belgium too had a substantial interest in Europa II, Mr Hanin said, but its government was never consulted. It is clear that when the conference does meet—probably in early July—a few explanations are going to be needed.

The ELDO staff in its statement condemns the attitude of the member states, and points out that the Europa project was killed before the organization's new structure—devised after the failure of F11 in November 1971—had had a chance. The staff "refuses to be associated with the responsibility for the waste of not completing a programme of such heavy capital investment, of which very little use can be made even if the L3S programme is carried out.

"It is clear that the failure of these programmes is primarily due to the political incapacity of the member states to define a common purpose and work together in a genuinely cooperative spirit," the staff says. The next launch had been scheduled for October 1, 1973, and the ELDO staff "protests strongly at the political failure of the member states being presented to public opinion as a technical failure discrediting their professional ability".

But while the ELDO staff and the Belgium government may be justifiably angry at the speed with which they were presented with a *fait accompli*, the Belgians are still eager to continue work on L3S, the launcher proposed by France. Mr Hanin, however, made it clear that he would be happier if the launched is managed by a European organization rather than by France.

ELDO, however, is most unlikely to manage the programme, even though the organization as such still exists, and may continue to do so until the new space agency comes into existence (see page 126). The Belgians will propose, however, that ELDO should organize the collection of the European money that will be put into the L3S programme if it goes ahead. But it has been suggested that the launcher might even be managed by the European Space Research Organization as a special project in the same way that ESRO manages the Spacelab programme.

SCIENCE RESEARCH COUNCIL

New Chairman

PROFESSOR S. F. EDWARDS was named last week to succeed Sir Brian Flowers as chairman of the Science Research Council in October. Professor Edwards, who is 45, is currently John Humphrey Plummer Professor of Physics at the University of Cambridge.

Professor Edwards will take full time leave of absence from Cambridge during his tenure as chairman of the SRC, which, in the first instance, will be for four years. He has also taken up an existing vacancy on the council until the time he becomes chairman.

Professor S. F. Edwards

Professor Edwards was educated at Bishop Gore Grammar School, Swansea, Gonville and Caius College, University of Cambridge, and Harvard. He also spent a year at the Institute of Advanced Study at Princeton in 1952 before taking up a post at the University of Birmingham. He moved to Manchester in 1958 and became professor of theoretical physics there in 1963. He has been a Fellow of the Royal Society since 1966 and a vice-president of the Institute of Physics since 1970. He has served the SRC in various capacities since 1968.

The retiring chairman and the new chairman of the council have had remarkably similar careers. Both were educated at Bishop Gore Grammar School in Swansea and at Gonville and Caius College at Cambridge. Both are theoretical physicists but with quite different interests — Sir Brian is a nuclear physicist whereas Professor Edwards' chief interests lie in chemical physics. Both have also held chairs at the University of Manchester and been on the staff of the University of Birmingham.

NEW WORLD

No Treaty Without Inspection

by our Washington Correspondent

SPOKESMEN for the Nixon Administration last week took an uncompromising stand on the need to include provisions for on-site inspection in any treaty banning underground testing of nuclear weapons. Their statements, made to the Senate Subcommittee on Arms Control, indicate that the Administration is not yet prepared to propose new initiatives to break the ten-year impasse in negotiations for a comprehensive test ban treaty.

But a number of other witnesses, including scientists, senators and the man who negotiated the partial test ban treaty in 1963, argued that the time is now ripe for an agreement banning all nuclear testing, and urged President Richard Nixon to pave the way for a formal treaty by seeking agreement with the Soviet Union for a mutual moratorium on underground tests. The forthcoming summit meeting between Mr Nixon and Mr L. Brezhnev, which will probably take place next month, would be an ideal time to announce such a moratorium, they suggested.

The focus of the hearings was a resolution introduced into the Senate earlier this year by Senator Edward M. Kennedy, and now sponsored by some thirty-three of his colleagues, which calls for a moratorium on underground testing and for President Nixon to offer fresh proposals for a comprehensive test ban (see *Nature*, **241**, 361; 1973). But Administration witnesses opposed the resolution last week because they believe that a treaty banning underground tests could not be adequately policed unless it contains a provision for on-site inspection to determine whether possible violations have taken place. That has always been the chief stumbling block in the path of negotiations for a comprehensive test ban treaty, for while the United States has been unrelenting in its insistence on on-site inspection, the USSR has been just as insistent that national means of detecting explosions—seismometers, surveillance satellites and other intelligence—would be sufficient to guard against violators.

In some respects, Administration witnesses last week took a firmer line on on-site inspection than they did during the last round of Congressional hearings on the test ban in 1971. Their argument is that in spite of significant progress during the past ten years, seismological techniques are not able to detect all low yield explosions and, perhaps more important, there are a number of seismic events each year which cannot be identified definitely as either explosions or earthquakes on the basis of their seismic signals.

Dr Jack Evernden, a seismologist with the Arms Control and Disarmament Agency, told the subcommittee that there would be "10 or so" events a year in the USSR with a magnitude of 4.0 or more on the Richter scale (equivalent to an explosion of one or two kilotons fired in solid rock) which could not be positively identified by national means. On-site inspection would be needed to determine the nature of such events, the Administration witnesses suggested, and Dr John S. Foster jun., Director of Defense Research and Engineering, flatly stated "with the inadequacies inherent in the seismic verification techniques, and which are likely to persist in any future system, it is necessary to supplement this technique with other verification modalities".

But that position was contested by several witnesses. In particular, Dr Herbert Scoville, former Deputy Director of the CIA, pointed out that very small events — below about 0.5 kilotons—would not be detected at all because their signals would be lost in the background seismic noise, and in those instances there would be no reason to inspect. He also said that the anomalous events which cannot be positively identified lie in a small range—between 0.5 and one or two kilotons—and a possible evader could not be certain that any specific test would remain unidentified. "It is clear," he said, "that on-site inspections serve almost no useful purpose in verifying a comprehensive test ban."

Dr Scoville and Dr Wolfgang K. H. Panofsky, Director of the Stanford Linear Accelerator, also pointed out that the US dropped its insistence on on-site inspection in a similar set of negotiations—those which led to the signing of

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AUTO EMISSIONS

Detroit Concedes

by our Washington Correspondent

THE prospect of a long fight between the automobile manufacturers and the Environmental Protection Agency over emission standards receded this week, when the four largest motor manufacturers announced that they will comply with the interim standards for 1975 vehicles, introduced as a compromise on April 11. The interim standards, less severe than those originally proposed, require that carbon monoxide and hydrocarbon emissions should be reduced by 83 per cent in the United States as a whole, and by 90 per cent in California. What the manufacturers now say is that the California requirements in themselves entail the use of catalytic convertors in the exhaust systems of new automobiles, and that they may just as well be ready to install the devices on cars intended for some other parts of the United States as well. Although it would have been possible to appeal against the imposition of the interim standards, the manufacturers said last week that they could not afford to risk the substantial delay that would have followed.

Argument about emission standards and their consequences is likely, however, to continue. Last week, the Federation of American Scientists put out, in its newsletter, a complaint against the use of catalytic convertors for reducing the amounts of pollution in auto emissions, especially now that the Environmental Protection Agency is preparing to set less ambitious goals for the emission of nitrogen oxides. The FAS statement echoes the commendation of the stratified charge engine put out last year by the National Academy of Science and asks that consumers should be protected from the risks that catalytic convertors will fail, sometimes without car owners knowing what has happened. The FAS is also concerned that the catalytic convertors entail greater fuel consumption, and asks not merely that consumers should be given objective information about the fuel consumption of new motor cars but also that the EPA should re-examine its policy on auto emissions so as to take account of the consequences for the fuel economy. It estimates that the use of catalytic convertors may entail an extra cost of $270 a year for each car.

the SALT agreement on ABM systems —and Mr Averell Harriman, former US Ambassador to the USSR and chief negotiator for the partial test ban treaty a decade ago, added that he believes the USSR would not sign an agreement which it intends to violate.

Those who argued in favour of the senate resolution suggested that the advantages of stopping nuclear tests would be outweighed by the disadvantages that may result from possible violations. One key question in such a determination is the military significance of small nuclear tests, and that, of course is classified. But Dr Panofsky suggests that the arms race is paced more by non-nuclear developments—delivery systems and so on — than by nuclear developments, and thus a ban on testing would not threaten US security. But Dr Philip J. Farley, Acting Director of the Arms Control and Disarmament Agency, said that "while seismic capability has improved, so has our understanding of the military potential of nuclear tests of lower yields". And Dr Foster said that there are some cases in which nuclear tests involving an explosive yield of a half or a quarter of a kiloton would be militarily important. But if that is the official position of the Department of Defense, it would be a serious blow to chances of ever securing a test ban treaty because such small events could never be detected by seismic means.

The Administration witnesses all reaffirmed the US government's commitment to achieving a comprehensive test ban, provided it could be adequately verified. But, unless their unwavering insistence on on-site inspection is simply an initial bargaining position for the next round of the Conference of the Committee on Disarmament to be held in Geneva next month — which some observers are suggesting—the chances seem dim that a comprehensive test ban treaty will be signed before the tenth anniversary of the partial test ban treaty.

COMMISSION

How to Run Universities

THE Carnegie Commission on Higher Education has now turned its attention to the problems of how to strike a balance between the conflicting interests in higher education (*Governance of Higher Education*, McGraw-Hill, April 1973) and has in particular blessed the continuation of the institution of academic tenure. The report says that the issue is more of a challenge now than ever, partly because of the past growth and increasing diversity of universities and colleges, the pressures generated by the slackened pace of growth now in prospect, the demands of students and the conflicting views among the faculties of universities and colleges about the function of higher education. The commission also includes enhanced public interest and reduced willingness to "accept authority" among the pressures on higher education. The problem, it says, is how to ensure a "reasonable" degree of independence from federal and state governments, separation from party politics, freedom for faculty members and faculty influence on academic affairs.

Among the commission's recommendations are the following:

- Where universities derive their support from public funds, there should be a general understanding between donors and recipients about the need that financial control should extend only to the outlines of academic policy. Budgets, for example, should not be defined in line-by-line items.
- Governing boards of publicly supported institutions should not include elected officials, especially those with financial powers, while the members of boards appointed, for example, by state governors should be screened in advance for suitability. At least some board members should be nominated for service by faculty, students and alumni.
- The commission has found that a majority of teachers in higher education is now in favour of collective bargaining and almost half of a representative sample considers that strikes may now be legitimate in certain circumstances. It asks that all colleges should conform to the standards defined by the American Association of University Professors in 1972, which assert the importance of collective bargaining in higher education but which also draw attention to the special needs of universities and colleges. Those colleges in which collective bargaining is at present outlawed should be reformed.

Alice in Eco-land Again

"THE Sierra Club magazine *Bonanza*," said Alice, "wants no more sheep to be raised because the sheep farmers are killing coyotes."

"Quite right," said the Mad Hatter. "The sheep must go. The coyotes are native Americans. They lived on the range land before the sheep came."

"But don't coyotes eat sheep?" asked Alice.

"They do," said the Hatter, "but mutton fat is very high in cholesterol. It is not healthy for elderly coyotes who are subject to coronary disease. They should eat deer and rabbits, which are rich in essential unsaturated fatty acids."

"What did you say?" asked the March Hare.

"I didn't mean you," said the Hatter "You are too tough."

"Where shall I be able to get wool to knit sweaters?" asked Alice.

"You might try shearing a coyote," said the Hatter. "They will become very tame when the sheep leave."

THOMAS H. JUKES,

University of California,
Berkeley, California

SPACE

Skylab in Trouble

THE entire future of the Skylab project hangs in the balance this weekend with the failure on Monday of this week of the solar panels on the space station. These panels, which should produce in all about 21 kilowatts of electricity, failed to open when signals were sent from Earth although four other solar wings which provide power for controlling the optical telescope attached to Skylab are working satisfactorily.

The Skylab crew was due to begin work in the laboratory on Tuesday, but following the failure of the power system the launch of their Saturn 1B was put off until next Sunday (May 20). This allowed the mission planners time to assess whether there was any point in sending up a crew to a spacecraft with only half power. One question which was asked was whether the crew could reactivate the solar panels once they had arrived. But Mr Walter J. Kapryan, launch director at Cape Kennedy, said on Monday that the astronauts could do nothing to correct any malfunction in the solar panels. In particular Mr Kapryan emphasized that the panels could not be made operational by a space walk.

There are no firm plans for any Skylab missions after the present one and it is unlikely that Congress will feel generous enough to provide NASA with increased funds if the present mission has to be aborted.

Partly because of this, there seems a good chance that NASA will proceed with a more limited Skylab mission even if the power fault is not rectified.

There are four chief objectives of the Skylab mission. First, it is meant to determine the ability of people to live and work in conditions of weightlessness for extended periods. Second, the mission is to make observations with the optical telescope which are not possible from Earth. Third, to develop improved techniques for surveying the resources of Earth from space. Fourth, other experiments which are of particular interest if carried out in zero gravity and outside the atmosphere.

NEWS AND VIEWS

Double Helix in Single Crystals

THE crystal structure of the dinucleotide phosphate adenosyl-3′,5′-uridine phosphate (ApU) described by Rosenberg *et al.* on page 150 of this issue of *Nature* can be regarded as the "missing link" in nucleic acid stereochemistry. The crystal contains two molecules per asymmetric unit which interact with each other to form a segment of double helix two base pairs long. Each adenine is paired with a uracil and the pairing is of the type proposed for DNA by Watson and Crick; the helix is right-handed with the two sugar phosphate strands running in opposite directions so that there is an approximate two-fold axis relating the two molecules in the asymmetric unit. This two-fold axis is, however, local to the asymmetric unit and is not a crystallographic symmetry element. The stereochemistry of the sugar-phosphate chain is very similar to that determined for a variety of synthetic and naturally occurring two-stranded RNAs from X-ray diffraction analyses of crystalline fibres.

The similarities between the torsion angles observed in single crystals of nucleic acid components, and those determined in fibre diffraction analyses of nucleic acids themselves have been noted by several workers. This analysis of ApU puts the final touches to such correlations by revealing a structure of a nucleic acid component which not only has the preferred torsion angles characteristic of nucleosides and nucleotides in single crystals but which also looks like a nucleic acid double helix. As the authors rightly claim, it is the double helix at atomic resolution.

Perhaps the most important feature of the structure of ApU is that the base pairing is of the Crick-Watson type. This is the type of base pairing proposed as the basis for molecular recognition in, for example, replication, transcription, codon-anticodon interaction, and for all the structures of naturally occurring and synthetic two-stranded nucleic acids analysed by X-ray fibre diffraction. It is not, however, the form observed in mixed crystals of adenine and thymine (or uracil) bases, nor in the mixed crystal of the nucleosides adenosine and 5-bromouridine. In these crystals pairing through hydrogen bonding of adenine with thymine is observed but it is of the "Hoogsteen" rather than the Watson and Crick type. If the Hoogsteen pairing had been observed in ApU no doubt the analysers of nucleic acid fibres could have retreated and regrouped, but it is a relief to know that this is not necessary.

With the many pearls offered so obviously in the ApU analysis it would be only too easy to overlook many other features of considerable interest. The unit cell contains twenty-six water molecules; when the final analysis locates these accurately their stereochemistry should contribute greatly to understanding of preferred patterns of hydration on nucleic acid double helices and hence to models for hydrophobic stabilization. The binding of ions to nucleic acids is of considerable biological and stereochemical interest. In ApU there is one sodium ion per molecule. The two ions in each asymmetric unit occupy distinct sites. One is complexed between phosphate groups, but the other lies in the minor groove of the DNA helix with octahedral coordination to two of the uracil oxygen atoms and to water molecules. Clearly this latter, rather unexpected type of interaction will stimulate a great deal of physico-chemical interest.

The structure of ApU is given added interest by the simultaneous determination in the same laboratory of the structure of the dinucleotide phosphate guanylyl-3′,5′-cytidine (GpC) (Day *et al., Proc. US Nat. Acad. Sci.*, **70**, 849; 1973). GpC also crystallizes as a double-stranded fragment but in this case the two-fold axis relating one GpC molecule to the other is a crystallographic symmetry element. The base-pairing is of the Watson–Crick type and, as for the structure of ApU, the conformation of the double-helical fragment is very similar to that determined by X-ray analysis of crystalline fibres of two-stranded synthetic and naturally occurring nucleic acids. Again the structure is heavily hydrated with thirty-six water molecules per unit cell.

These studies emphasize the value of single crystal studies of monomers and oligomers in the structural analysis of macromolecules. Increasingly it is being recognized that not only covalent bound geometry but also orientations about single covalent bonds and patterns of intermolecular interaction are strikingly similar in single crystals of macromolecular components to what they are in the macromolecules themselves. As a consequence very precise information obtained from highly refined structures of small molecules can be used in stereochemical analyses of large molecules in which attempts are made to correlate biological, chemical and physical information within a single molecular model.

W. F.

Invoking Black Holes

ELEMENTARY accounts of the scientific method often stress the way in which the observations of some unexplained object are built up so that a theoretical model can be inferred from them, as for example in the way the neutrino was invented to explain observations of β-decay. But there are spectacular counter-examples in which the solution came first and the problem which it fitted was found afterwards. The way in which the positron was found to fit the Dirac theory springs to mind. An astronomical example might be the neutron star. This object was shaken by Fritz Zwicky from what he called his morphological astronomical kaleidoscope, a way of permuting all possible combinations of matter into new objects. It was also invented by Oppenheimer and Volkoff in 1939 as a simultaneous solution of the equation of hydrostatic equilibrium of a self-gravitating mass and an equation of state for a degenerate material made up of neutrons. It was more than 30 years before an astronomical problem was found which appeared to require the neutron star for its solution—the radio and X-ray pulsars.

Of these the X-ray pulsars have provided the most clues to their nature, for it is found, by observing the Doppler shifting of their periods and the way in which they are regularly eclipsed, that they are components of double star systems of small separation. In at least one case, known as Hercules X-1, the second star of the system is also known as an optically variable star, HZ Herculis. It has thereby become possible to deduce the mass of the X-ray pulsar in this system, which turns out to be about 0.5 of a solar mass. The significance of this result is that the simultaneous solution of the equations of hydrostatic equilibrium and of state for degenerate fermions is possible only for a restricted range of self-gravitating masses, whose upper bound (known as the Chandrasekhar limit) lies near 2 solar masses. The observed mass of the Hercules X-1 pulsar falls within the allowed range and gives confidence in the idea that it is a neutron star.

Not all X-ray binary stars can be described as confidently as this. Cygnus X-1 is the prototype of the difficult ones. Its soft X-ray flux was discovered by the Uhuru satellite to vary rapidly, but not periodically like a pulsar. The rapidity of the variations extends at least to the resolution time of the Uhuru satellite, namely 56 milliseconds. It is often said that this proves that the star responsible for the X-ray variations must be less than 56 light-milliseconds across, that is, less than 0.03 solar radii. If the X-radiation comes from gas, gravitationally compressed as it falls to a star's surface, and heated to X-ray emitting temperatures, as was proposed generally for galactic X-ray sources by Burbidge and Prendagast in 1967, then the X-ray emitting region envelopes the star and the star is indeed small. But the X-ray emitting region may actually be localized, tied to a star of arbitrary size.

Can one say anything more definite about this star? Cygnus X-1 is identified optically with a binary star system known as HDE226868, of which only one component is seen. It is a B-type supergiant star. The mass of its invisible companion is more than 10 solar masses, far above the Chandrasekhar limit. The companion is therefore not a self-gravitating mass of fermions, neither a white dwarf nor a neutron star. There are, in fact, normal stars of this mass, whose light would be swamped by the large output from the supergiant, but if one is committed to the idea that the X-radiation is from compressed gas falling from the supergiant onto the invisible companion, one is forced to exclude a normal star as not having an intense enough gravitational field, and to speculate that the companion is a black hole. Indeed, as a lone black hole is one of the most difficult of all objects to detect, predictions about their appearance have concentrated on their effect on surrounding matter, so that it was pointed out by Shklovsky in 1967 that in the search for a black hole, one would look for a star affected gravitationally by an unseen companion, close enough to draw in matter from the normal star and emit X-rays. This could be a description of Cygnus X-1, published several years before its details were known, and it is not surprising that speculation about the nature of Cygnus X-1 has proceeded on the assumption that it is the first known example of a black hole.

As this account should have brought out, however, this conclusion is based on the facts interpreted by the theory of gravitationally compressed gas as the source of X-ray emission. Alternative models must be examined, if only to play the devil's advocate in testing the right of Cygnus X-1 to be called a black hole.

Such an alternative model was proposed by Bahcall, Kulsrud and Rosenbluth in *Nature Physical Science* last Monday (**243**, 27; 1973). They propose that the Cygnus X-1 binary system consists of two normal stars whose magnetic fields are interlinked and wound up by their orbital motion. The magnetic field breaks and reconnects at weak points with energy dissipation as X-radiation and so on, in a manner reminiscent of solar flares. As in the idea of gravitational compression, the X-radiation is powered by the gravitational field of the stars, so there is plenty of energy available for conversion, but the radiation arises from local regions in the magnetosphere of the binary star system. Random bursts of emission from these regions would cause the fluctuations observed in the X-ray flux.

If this model is substantiated, perhaps by the two observational tests which the authors themselves propose, then the problem to which black holes are an eager solution has yet to be found.

From a Correspondent

NUCLEIC ACIDS

Sweat and Serendipity

from our Molecular Biology Correspondent

ONCE more Sanger has produced an ace from his sleeve, and with that effortlessly inexorable air that suggests—as Disraeli in his exasperation said of Gladstone—that God had personally secreted it there. He describes with his colleagues (Sanger *et al.*, *Proc. US Nat. Acad. Sci.*, **70**, 1209; 1973) a freshly minted method for sequencing DNA, and its application to a bacteriophage. The luck that attended their first foray into the sequence of R17 phage RNA has not deserted them, for they seem again to have hit on an interesting region between two cistrons; they report the sequence of a tract of fifty nucleotides, with of course another fifty on the opposite strand thrown in for nothing.

The new methods depend on first making a primer for copying the minus strand of the DNA, using the plus or "sense" strand as template, with DNA polymerase of *Escherichia coli*. Taking advantage of the known sequence of the principal coat protein, an oligodeoxynucleotide containing the codons for a presumed initiation point was synthesized (by what are now well established, if exacting, synthetic routines). With f1 phage DNA as template, the octanucleotide primer starts the synthesis of a new chain, the first eight nucleotides of which are thus known by definition. The next step was to omit from this reaction mixture one or more of the nucleoside triphosphate monomers. The oligomeric product is then separated by chromatography on ion-exchange paper, using Sanger's "homochromatography" technique, whereby oligonucleotides are displaced according to size by a front of mixed fragments of a non-radioactive digest sweeping through them, in the second dimension. The spots are subjected to nearest-neighbour analysis and depurination to determine their sequence, though the direction of displacement of each successive oligomer spot relative to that of its neighbour in the chromatogram practically allows the sequence to be read off by inspection. If only ATP and TTP are present in the reaction mixture, no further products appear after addition of six nucleotides to the primer. It follows that the seventh must be a G or C. If CTP is included, another six nucleotides are added before the presence of a G in the sequence brings the polymerization to a stop.

Beyond this point a different technique has to be applied, and here Sanger and his colleagues make use of an observation of Berg's that the DNA polymerase in the presence of manganese in place of magnesium will incorporate ribonucleotides. If then, for example, riboCTP is included in the monomer mixture, the product will contain a ribose wherever a C occurs in the chain, and will accordingly be labile to pancreatic ribonuclease at each of these points. With f1 DNA, in the chosen conditions, this strategy produces seven prominent ribonuclease fragments, which were sequenced by the methods developed earlier. Since the enzyme does not run at uniform rate along the sequence, the polymerization product at a given time contains segments of different lengths, each terminating in a residue that the enzyme finds relatively unpleasing. These products were separated by the "homochromatography" method, and analysed for their content of ribonuclease fragments. This allowed the order of the seven fragments to be established, and so the sequence of the first fifty residues synthesized by the enzyme.

The first fact to emerge is that initiation had not occurred at the expected point, and indeed there is some evidence of an error in the reported coat protein sequence at this position. Rather, the fifty residues appear to encompass an intercistronic region in the complementary sequence, which is also that of the messenger, and which contains three termination codons, all mutually in phase, just as was found at the end of the coat protein cistron of R17 phage RNA. On either side would be the message for a protein ending in gly-phe-ile-ala, and another beginning formylmet-val. This reading, of course, is not yet proven, for the phase could be taken differently. A feature of the nucleotide sequence is that it is remarkably rich in T residues, and contains several short repeated sequences, that gives support to the theory whereby the early evolution of DNA involved sequence duplication, followed by mutations to generate useful messages. There is a notable homology in part of the f1 sequence with that of an icosanucleotide from øX 174 DNA, which represents a ribosomal and binding site, and contains the initiation sequence for a structural protein.

Meanwhile the precepts of Sanger and his associates are being applied to many of the central problems of molecular biology. Murray (*Biochem. J.*, **131**, 569; 1973), working with λ and 424 phage DNA, has used the method of radioactive end group labelling with polynucleotide kinase to obtain striking nucleotide maps from these species. Many oligonucleotides were sequenced, and the functionally important terminal sequences were determined. The two phages were found to be identical in this respect, which provides the physical basis for their ability to form mixed dimers by way of their cohesive unpaired ends.

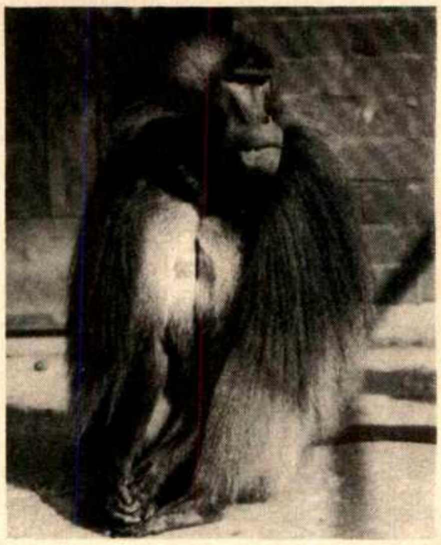
On the RNA front, Rensing (*ibid.*, 593) has produced a new sequence of seventy-three residues from R17 RNA, which is not related to the coat protein genes. It can be read through in one frame from end to end, and is remarkable in that it contains two runs of twenty-three and twenty-four residues, the sequences of twenty-two of which are identical. What it codes for is not established, for the sequences of both the A protein and the polymerase are unknown, and only the coat protein, the RNA sequence for which is now fully determined, has a known sequence. In a further report, Rensing *et al.* (*ibid.*, 605) give the sequence of another fragment of thirty-three nucleotides, as well as two other pieces identified as arising respectively from the start of the A-protein cistron and just before the ribosome binding site that precedes the coat-protein cistron. One of the fragments especially has the potential to form a hairpin of 11–12 successive base pairs.

PRIMATES

Fossil Baboons of Africa

from a Correspondent

THE gelada baboons of Ethiopia are no more than a surviving remnant of a once-thriving group of specialized grassland monkeys, according to Jolly, who has made an exhaustive study of large African Plio-Pleistocene fossil baboons generally attributed to the genus *Simopithecus* (C. J. Jolly, *Bull. Brit. Mus.* (*Nat. Hist.*) *Geol.*, **22**, 1; 1972). Indeed, Jolly feels that these fossil forms should be placed as a subgenus in the same genus as the living gelada baboon (*Theropithecus gelada*), as an indication of their close relationship. This suggestion is based on the hypothesis that both the fossil *Simopithecus* and the modern geladas share many adaptations for an open-country grassland niche, with grass blades, seeds and rhizomes—picked by hand—providing the bulk of the diet. *Simopithecus* is thought to have resembled the gelada in that it rarely, if ever, utilized trees, because study of various limb fragments indicates extreme adaptation for terrestrial locomotion (short, stubby digits; robust skeleton; poorly developed hallux; arms and legs similar in length).



Gelada baboon (photo: Zoological Society of London).

The general picture of a large bodied, ground-living form specialized for feeding on small objects is confirmed by detailed study of the skulls, jaws and teeth of *Simopithecus*, which are richly illustrated in Jolly's monograph. When compared with typical baboons (genus

Testosterone and Ovarian Function

WITH the development of mammalian genetics the growing range of mutants available is becoming valuable for answering fundamental problems outside genetics. In *Nature New Biology* next Wednesday (May 23) there is a further report on the testicular feminization or *Tfm* mutant of the mouse, which in the hands of Ohno and his colleagues has already proved of great interest.

This X-linked gene is interpreted as a non-inducible mutation of the regulatory gene involved in the response of target organs to testosterone, and animals affected with it lack all response to androgen. The affected animals are usually genetically male, *Tfm*/Y, and have testes. Ohno *et al.* have now found six affected animals which were chromosomally XO, that is *Tfm*/O, and therefore genetically female and with ovaries. This has made it possible to test whether testosterone has any function in female reproduction. The ovaries of the youngest *Tfm*/O were normal, but the five older animals showed rapid deterioration of the ovaries, best described as precocious luteinization of follicles. It thus seems that in female mice a response to testosterone is essential for normal ovarian function.

The X linkage of the *Tfm* gene is of further interest in this context, because mammalian oocytes also require the presence of two X chromosomes for completely normal survival. Could the normal allele of the *Tfm* gene be one of the genes whose function in double dose is needed? The degeneration in *Tfm*/O was indeed similar to, but more severe than, that seen in +/O ovaries. Further work will, however, be necessary to answer this question definitively, because the work of Ohno *et al.* does not yet exclude the possibility that the effect in *Tfm*/O ovaries was more generally systemic, involving hormonal interactions and the hypothalamo-pituitary axis.

Papio), *Simopithecus* and modern geladas show a complex of characters which indicate extensive adaptation to grass eating: the cheek-tooth crowns are more complex, and the molars and premolars have a relatively large surface area, whereas the incisors (used for extensive food preparation in *Papio* species) are very small. The lower jaw has a high, vertically ascending ramus, and the contours of the braincase indicate well-developed temporal musculature for mastication. Finally, there is some indication that the third molars may have erupted relatively late in *Simopithecus*, thus extending the active life of the cheek teeth.

Jolly shows, however, that it is misleading to refer to all *Simopithecus* as "giant baboons". The two recognized species, *Theropithecus* (*Simopithecus*) *oswaldi* and *T.* (*S.*) *darti*, both exhibit a fair range of body size; the smallest known individuals are comparable in size to modern *Papio anubis* (olive baboon), and the largest known fossil males were probably as big as a female gorilla. As in the modern gelada, there was considerable sexual dimorphism, and males were apparently considerably heavier than females.

Apart from providing a detailed account of the known fossil material attributable to *Simopithecus*, Jolly also considers the geographical range and timespan covered by the fossils. *Simopithecus* was obviously widespread in East and South Africa during the late Pliocene and the bulk of the Pleistocene, occurring at presumed "waterside" sites. The earliest known fossils date back to about 5 million years ago, and extinction of the fossil subgenus seems to have occurred about 50,000 years ago. This disappearance of a once-successful species is traced to human hunting and ecological competition. Jolly postulates that the modern gelada represents a highland branch of a Pliocene ancestral stock which also gave rise to lowland forms of the subgenus *Simopithecus*. At some time in the late Pleistocene, human activity may have led to the extinction of the large-bodied lowland forms, leaving the highland geladas (temporarily?) relatively unscathed. Jolly does not believe that overhunting led directly to the extinction of *Simopithecus*. Instead, he suggests that human activity upset the delicate balance of ecological separation between *Simopithecus* and other baboons (*Papio* species), and this in turn led to the extinction of the grass-eating *Simopithecus*.

Jolly's account attains a wider significance in that the dental adaptations for small-object feeding in *Simopithecus* and the gelada may provide a model for dietary adaptations associated with dental changes in the early stages of human evolution (Jolly, *Man* (*Lond.*), NS, 5, 1; 1970).

NUTRITION

How Much Vitamin C?

from a Correspondent

FOR the past several years Linus Pauling has vigorously maintained that people benefit, indeed need, perhaps more than ten times the minimal daily requirement of vitamin C that was established many years ago, if they are to be really healthy. He has in particular argued that by taking what are by conventional standards very large daily doses of ascorbic acid people can avoid such diseases as the common cold and influenza. As Yew points out in the current issue of the *Proceedings of the National Academy of Sciences* (**70**, 969; 1973), sceptical nutritionists "who ignore this suggestion or have merely used rhetoric to refute it appear to accept axiomatically two crippling but time-honoured *assumptions*, first, that ascorbic acid functions merely to prevent 'scurvy' and, second, that within a species a narrow range of needs must *of necessity* exist".

Nobody of course denies that the current recommended daily minimal doses are insufficient to prevent scurvy; the question is whether a much larger dose not only prevents scurvy but also promotes good health and development of human beings. Yew claims that very little information about the amount of ascorbic acid needed to keep humans healthy has been obtained in the 41 years since the vitamin was identified. In an attempt to obtain some comparative data he fed a scorbutic diet supplemented with ascorbic acid at four different levels (0.05, 0.5, 5.0 and 50 mg/100 g of body weight per day) to populations of healthy guinea-pigs. He then measured the response of animals eating these various amounts of ascorbic acid to surgical stress, recovery from anaesthesia and scab formation and wound healing. His conclusion is that young guinea-pigs need about 5 mg per 100 g body weight per day and under stress the requirement is even higher. These amounts are much higher than anybody has supposed, even though commercial guinea-pig chow contains enough vitamin C to give animals about 3.3 mg per 100 g body weight.

It is perhaps naïve simply to extrapolate from these data to the human need for this vitamin, but if the extrapolation is made a 30 kg child would need 1,500 mg of ascorbic acid daily compared with the US Food and Nutrition Board's recommendation of 40 mg per day. Yew's point is simply that it is now up to those responsible for setting vitamin requirement standards to get to grips with the problem and try and discover just how much vitamin C young people do need to develop to their full potential. He repeatedly throws out this challenge saying, "The burden of proof must now shift to the medical scientists who are really concerned about the health and physical condition of young people to show that considerations that apply to young guinea-pigs do not apply to young people." And broadening the issue Yew concludes that "if this gross uncertainty in human requirements for ascorbic acid prevails in nutritional science can there not be comparable uncertainty with

A Three Point Landing

MUCH scientific sleep has been lost over the problem of how transfer RNA binds to the aminoacyl synthetases and wherein their specificity to these enzymes resides. An attractive answer to these problems is provided by Dube in next Wednesday's *Nature New Biology* (May 23).

Dube, working with *Escherichia coli*, isolated complexes of $\mathrm{tRNA_f^{met}}$ and the methionyl-tRNA synthetase and treated these with ribonuclease T_1. Fingerprints of the resulting digests revealed that four oligonucleotides were present but in low yield in comparison with uncomplexed tRNA. These oligonucleotides could be unambiguously assigned to the anticodon loop, and the 3′ side of the anticodon arm, the extra arm and to the 3′ side of the stem. It was inferred that binding to the synthetase protected certain G residues in these regions of the structure from attack by the enzyme.

This conclusion suggested to Dube that these regions were on the same side of the molecule as opposed to the TuC and DiHU loops. This suggestion fitted in nicely with the structure of $\mathrm{tRNA^{phe}}$ proposed by Kim *et al.* (*Science*, **179**, 285; 1973) from X-ray crystallographic evidence. The anticodon loop and the 3′ end are at the two extremities of an L in this model, and if the enzyme is also to interact with the extra loop it must have three binding sites located on an arc which fits into the angle of the L. Recent work by Schulman (*Proc. US Nat. Acad. Sci.*, **69**, 3594; 1972), who chemically modified G_{46} (in the extra loop), G_{71} (in the 3′ stem) and C_{35} (in the anticodon loop), supports this notion, for these modifications led to complete inactivation of $\mathrm{tRNA_f^{met}}$ with respect to methionine acceptance. It is of interest that these residues are conserved in $\mathrm{tRNA_m^{met}}$ which is aminoacylated by the same enzyme.

Dube finally suggests that a three point attachment to an arc shaped active centre may be the key feature in tRNA recognition and that the variable extra arm may play a vital part in this process.

respect to other vitamins, such as vitamin A and vitamin D?" Yew at least is firmly in Pauling's camp and it will be interesting to see what response there is to his provocations.

MOLECULAR BEAMS

Scattering Branches Out

from a Correspondent

Members of what was described by Professor D. R. Herschbach (Harvard University) as the "lunatic fringe", who first reported molecular beam scattering results at the Faraday Discussion on inelastic collisions in 1962, were in a confident mood at the Chemical Society Faraday Division general discussion on molecular beam scattering held in University College, London, from April 16–18. This confidence stemmed from new techniques which make it possible to study some of the problems in chemical reaction and energy transfer which arise in the main stream of chemistry. These developments include the liaison, if not yet the marriage union, of spectroscopic and scattering methods, as in the laser-induced beam fluorescence experiments described by Dr R. N. Zare (Columbia University), as well as the reports from many laboratories describing experiments with a broad range of atomic and molecular species. This latter feature was in particular contrast to the position at the last Faraday Discussion on this topic in 1967 when beam experiments had to include at least one alkali atom because of detection difficulties with other species.

The measurement of intermolecular forces by beam methods was one of the topics discussed at the conference; interest here is now centring on the forces between energetically excited or otherwise chemically labile species. Unfortunately there are few quantum computations of such potential surfaces with which these measurements may be compared.

Professor J. P. Toennies (Max-Planck Institute, Göttingen) described measurements of the angular distribution of rotationally excited H_2 resulting from collisions with fast Li^+ ions. The distribution of rotational states was resolved by measuring the associated change in the velocity of the Li^+ ion using a time-of-flight method. This is a new achievement for, until recently, techniques were insufficiently sensitive to permit any information about the angular distribution to be obtained. Interestingly, the results presented were not in accord with the rather precise quantum calculations available for this system.

Most of the meeting was, however, concerned with the use of molecular beam methods to explore the dynamics of chemical reaction. Professor D. R. Herschbach (Harvard University) discussed the results of experiments involving four participating atoms such as $CsBr+ICl \rightarrow CsCl+IBr$. Depending on the precise reactants, the angular and energy distributions found for the product varied from those expected from a long-lived collision complex decaying with a statistical distribution of the energy into the product molecules, to those characteristic of a specific short-lived interaction. The transition between these two types of behaviour correlate with the shape of the complex predicted by molecular orbital theory. Reaction by way of a long-lived complex was also reported for systems such as $F+C_2H_4 \rightarrow C_2H_3F+H$ (Dr Y. T. Lee, University of Chicago). These were especially interesting because the energy distribution did not follow that expected statistically from the RRKM model for unimolecular reactions. These results suggest that the assumption inherent in this model, that chemical reaction is slow by comparison with a rapid internal randomization of the reaction energy in the complex, may be incorrect. This possibility was discussed by several speakers and the suggested use of information theory methods to characterize the degree of non-statistical behaviour found favour. Further light on this problem came from Monte Carlo trajectory calculations extended over the life of four-atom complexes (Na Br KCl); for this strongly coupled system, the statistical approximation seemed faily good.

The reaction between alkali metal atoms and alkyl halides, a molecular beam "chestnut", continued to be of interest to theoreticians and experimentalists alike, the latter still comfortably in the lead. The quantity of information available concerning these reactions is now so large that it is extremely difficult to find potential surfaces capable of reproducing, using Monte Carlo calculations, all the observed features. A successful surface would now be almost uniquely constrained.

The conference concluded with a summary contribution from Dr J. C. Polanyi (University of Toronto), who related present achievements and future prospects to the pioneering work in the 1930s of his father, M. Polanyi, who was present in the audience.

TECHNIQUES

Archaeological Dating

from a Correspondent

Subjects of interest to both archaeologists and scientists were discussed at the meeting on archaeometry and archaeological prospection in the Clarendon Laboratory, University of Oxford, from March 29 to 31.

Descriptions of new developments in instrumentation for geophysical survey based on electromagnetic and resistivity principles were described emphasizing the greater sensitivity and general portability now attainable. Methods of rapidly displaying the results of the measurements for archaeological evaluation were explained by Drs E. T. Hall (University of Oxford) and I. Scollar (Bonn Museum) who showed the desirability of presenting data as punched paper tape suitable for use with a computer programmed for pattern recognition and/or image enhancement.

It was natural, at Oxford, that much

Messenger RNA Activity in β-Thalassaemia

Human β-thalassaemia has been characterized by decreased or absent synthesis of the β-globin chain in erythroid cells and the defect has been localized at the polysome level. In *Nature New Biology* next Wednesday (May 23) Dow *et al.* report their studies of the activity in a cell free system of mRNA isolated from polysomes or whole cell lysates of the blood of patients exhibiting different levels of β-chain synthesis.

Using a Krebs II ascites cell free system with rabbit reticulocyte ribosomal initiation factors, Dow *et al.* demonstrate that addition of 10S RNA isolated from polysomes of non-thalassaemic cells led to the synthesis of both α- and β-chains in relatively equal amounts (α/β ratio of 0.79–1.0). This compared well with the α/β ratio of whole blood cells. When, however, 10S RNA from polysomes of β-thalassaemic blood was tested in the cell free system, a marked decrease in β-chain synthesis occurred (α/β ratios 1.8–3.6). Again the ratios were similar to those obtained with whole cells. The results were the same when 10S RNA was extracted from whole cell lysates rather than polysomes. Because the ratio of α/β chain synthesis is the same whether extracted RNA or whole cells are used, it is probable that mRNA, if present in β-thalassaemic cells, is either decreased or abnormal.

Previous workers who arrived at this same conclusion used a pool of cells of two β-thalassaemic patients or the cells of siblings or an individual patient as the source of mRNA. Dow *et al.* now confirm this result in a number of unrelated patients exhibiting a range of β-chain synthesis including no β-chain formation.

time was devoted to the varied aspects of thermoluminescence (TL) dating. In a critical review of the theories of the phenomenon Professor G. J. Garlick (University of Hull) pointed out that in fundamental studies difficulties in deducing trapping parameters have arisen and that non-thermal leakage of trapped carriers has been found. This leakage, which is caused by overlap of wave functions of trapped carrier state and ground state, affects the limits of dating by this technique. The intensive investigation of TL dating, which promises so much, was shown by several contributors. A rather large array of factors, for at least some of which corrections must be made before one can reduce the errors of TL dates to small proportions, were presented, but the overall impression was that the difficulties would be overcome. Dr A. G. Wintle (University of Oxford) reported that feldspars, zircon and fluorapatite all lose some artificially induced TL from the high temperature part of the glow curve after some days storage. Fortunately, quartz and limestone do not exhibit this "fading" effect. Dr V. S. Desai (University of Oxford) showed that pottery saturated with water attenuates the escape of radon, and subsequent members of the uranium-238 decay chain, the isotopes of which are responsible for an appreciable fraction of the radiation dosage received by the pottery. Thus, changes of water content and the porosity of the pottery could modulate the dose rate received as was pointed out by Dr V. Mejdahl (University of Copenhagen); and Dr J. Huxtable with Dr M. J. Aitken (University of Oxford) demonstrated that the radioactive impurities themselves could be leached in slightly acidic conditions giving yet another potential source of error in pottery during centuries of burial.

The magnitude of the supralinearity correction routinely applied during TL dating by the fine grain method was shown by S. J. Flemming (University of Oxford) to vary, in many instances, with the pottery's previous radiation history, and consequently affects the error in the date. Dr D. W. Zimmerman (University of Washington, St Louis) explained how these errors could be eliminated in certain special cases. He required that the inclusion in the archaeological ceramic itself was highly radioactive, as opposed to the normal case of matrix radioactivity. The zircon or apatite should have a sufficiently large internal dose rate of α-particles to swamp any environmental γ-dosage, and this also would not be attenuated by the presence of water. Such a specimen may be used for authentication purposes even after the pottery has been X-rayed in previous analyses, a process which would probably invalidate the standard TL authentication methods.

Dendrochronology was discussed at some length during one session. The method is relatively new in Britain though studied for some 70 years in the United States. Dr J. M. Fletcher (University of Oxford) presented the results of his two years' work on a series of oak trees, supplemented by timber from nearby mansions, panels from Tudor portraits and some large chests in Westminster Abbey. He has been able to construct a reference tree-ring curve back to AD 850. Similar impressive work by the palaeoecology laboratory at the Queen's University, Belfast, was described by Dr A. G. Smith. In four years they have assembled a series of floating tree-ring chronologies extending for about 7,000 years as attested by radiocarbon dating. This was accomplished by cross-linking oaks and pines preserved in some of the peat bogs in Ireland. It is hoped that the missing sections will soon be discovered and then a chronology rivalling that of the bristlecone pine will become available.

A spirited discussion following two contributions on methods of correcting radiocarbon dates ensued and it was postulated that two "types" of calibration curve were possible, one for explaining geophysical perturbations of atmospheric radiocarbon and another for the correction of archaeological dates. Dr H. McKerral (Scottish National Museum, Edinburgh) examined in detail corrections in the early bronze age of Britain and concluded that corrected dates were much later than expected on historical grounds. A statistical investigation by Professor C. Renfrew (University of Southampton) and Dr R. M. Clark (University of Sheffield) of bristlecone pine calibrated and historically dated Egyptian samples indicated that the errors of the radiocarbon dates had been underestimated or that the assigned calendar dates of some of the samples were in error, or both. Calibration curves fitted by computer indicated, however, that the Egyptian and the bristlecone pine data are compatible within the relevant time period. The fear initiated among some archaeologists by a "red herring" in a recent review article, that the bristlecone pine calibration data might be distorted by as much as 1.5 per cent by carbon-14 produced *in situ* in trees growing at high altitude by the action of cosmic ray neutrons with nitrogen fixed in the cellular structure of the wood, was allayed by Drs D. Harkness (Scottish Research Reactor Centre, East Kilbride) and R. Burleigh (British Museum) who reconsidered the theoretical predictions. They suggested that the amount of radiocarbon thus produced is below the limit of measurement, and so could not affect the calibration curve.

A New Protein Controlling Cell Division

IN *Escherichia coli* the completion of DNA replication is a prerequisite for cell division. It is known that protein synthesis is essential for cell division and, to date, two distinct periods of protein synthesis have been described, the first being required for the initiation of replication, the second taking place concurrently with replication. In *Nature New Biology* next Wednesday (May 23), Jones and Donachie describe a third period of RNA and protein synthesis which occurs at or subsequent to completion of DNA replication.

Jones and Donachie used a strain of *E. coli* which is able to resume RNA synthesis after treatment with rifampicin and removal of the antibiotic from the medium. They pretreated their cells in such a way that they became synchronized with respect to a single round of DNA replication and a single subsequent wave of division. Readdition of thymine after pretreatment led to a synchronous reinitiation of chromosome replication within 10 min. The replication was complete by 40 min and was followed 5 min later by cell division. Inhibition of completion of the first round of chromosome replication prevented cell division.

Addition of rifampicin up to 35 min after administration of thymine prevented most division but addition at 45 min had no effect on division. Addition of chloramphenicol at any time up to 40 min after thymine prevented all cell division. Later addition of chloramphenicol had no effect. Thus RNA and protein synthesis are required 5–10 min before division. RNA synthesis is not required at an earlier time, because if rifampicin is added at the same time as thymidine and subsequently removed (before the 5–10 min prior to division), the timing of division is unaffected. Furthermore, synthesis of this specific RNA and protein required for division can take place only after the replication of a late segment of the chromosome. Once fermentation has occurred, RNA and protein synthesis required for division can take place even after a 30 min delay.

These experiments therefore demonstrate the existence of a "termination protein" the synthesis of which is signalled by the completion of DNA replication and the function of which is presumably to act to prevent cell division before completion of chromosome duplication.

Great interest was shown in the description of Dr E. W. MacKie (Hunterian Museum, Glasgow) of his excavation of a stone platform near the large standing stone at Kintraw, Argyll. This, according to the theories of A. Thom, was the observation point of the megalithic midwinter solstice observatory in prehistoric times. Unfortunately, no artefacts were found but a petrofabric analysis of the stones of the platform is demonstrably similar to a platform known to be man-made and quite dissimilar to natural ones.

SOLID STATE

In Two Dimensions

from a Correspondent

A CALCULATION by Novaco (*Phys. Rev. A*, **7**, 678 ; 1973) provides strong support for an earlier suggestion that a single layer of helium atoms, adsorbed on a smooth surface at low temperature, behaves as a two-dimensional solid.

An adsorbed atom (adatom) is strongly bound to the surface of another material (substrate) by the so-called Van de Waals force of attraction. The adatom therefore finds itself trapped in a two-dimensional world where, if the substrate is smooth, it is entirely free to move in any direction parallel to the surface, but where motion in the third dimension, perpendicular to the surface, is rigorously forbidden. When many adatoms coexist on the same substrate, the normal concepts of kinetic theory may be expected to apply: each atom will possess a certain average speed, dependent on the temperature, and atoms will frequently collide with each other, exchanging energy and momentum ; but all these processes will be occurring in two dimensions rather than the normal three. The sole function of the substrate is that of constraining the assembly to remain in two dimensions, it being regarded otherwise as completely inert.

Several different substrate – adatom combinations have now been studied, but particular attention has centred on helium adatoms because the Van de Waals force between a pair of helium atoms is so small that a low density assembly can be expected to behave as an ideal two-dimensional gas, a situation which is relatively easy to tackle theoretically. At higher densities, the adatoms, being more closely packed on the substrate, will have less freedom to move about, and so might perhaps be expected to form a two-dimensional solid. This latter point is by no means obvious, however, for three-dimensional helium does not solidify, even at zero temperature, unless a substantial external pressure is applied.

Very striking demonstrations of the general validity of these ideas have been achieved by Dash and his colleagues at the University of Washington, who have measured the heat capacity of helium adsorbed on a form of graphite at temperatures of a few K. This substrate has the advantage, in addition to being very smooth, that a small volume of the material presents an exceedingly large area of surface for adsorption: their 12 cm^3 cell contained more than 200 m^2 of graphite surface. The heat capacity of the substrate was therefore considerably less than that of the adsorbed film, which could then be measured with impressive precision. In earlier investigations they were able to demonstrate that, at low (that is, submonolayer) coverages, the helium atoms indeed display many of the properties predicted for two-dimensional gases, but their most recent work (Bretz, Huff and Dash, *Phys. Rev. Lett.*, **28**, 729 ; 1972) concerns the situation where the substrate is, in effect, completely covered with helium. To their surprise, they found that the heat capacity passed through a very sharp maximum at a temperature which depended on the two-dimensional density of the monolayer.

They tentatively attributed this phenomenon to a melting process: at temperatures well below that of the peak, the atoms would all be localized in some sort of two-dimensional lattice ; whereas, above the peak, they would be able to move about freely, forming a two-dimensional liquid. Just as in the case of three-dimensional helium, the melting point would move to higher temperatures if the density of the two-dimensional solid was increased by adding extra adatoms, consistent with the experiment. A surprising feature of their results was that, at temperatures well below the peak, the heat capacity of the two-dimensional solid corresponded to that of three-dimensional helium at a pressure of several hundred atmospheres. This seemed remarkable at first sight, for the "actual" pressure (that is, that of the real three-dimensional vapour in equilibrium with the adsorbed film) was negligibly small. A plausible explanation was put forward, however, in terms of the very strong attractive force between adatom and substrate, which might be able to pull many additional helium atoms down onto the surface even though it meant compression of an already complete monolayer.

Novaco has now put this suggestion to the test, theoretically, by calculating the densities and corresponding heat capacities which would be expected at very low temperatures, if the monolayer were indeed a close-packed two-dimensional solid. That his calculated values are in satisfactory agreement with experiment is encouraging evidence in support of the model. Unfortunately, the heat capacity measurements yield no information on the two-dimensional lattice structure and, indeed, cannot show unambiguously that the monolayer is solid rather than liquid. Novaco points out that a neutron scattering experiment, now in the planning stage, may perhaps yield definitive answers to these questions.

Radio Emission from Supernova 1970g

SUPERNOVA 1970g was a type II event which occurred in the external galaxy M101. In next Monday's *Nature Physical Science* (May 21) Goss and colleagues report observations of the supernova remnant made with the Westerbork Synthesis Radio Telescope (WSRT) at 21 cm and 6 cm and with the Max Planck 100-m instrument at 2.8 cm.

The observations were carried out during late 1970 and early 1971. Mapping at 21 cm took place during November, December and January ; the resolution of the final map is 25 arc s × 30 arc s. Better resolution is achieved at 6 cm, of course, but at that wavelength the source was viewed only for one period of 12 h on January 26, 1971. But this observation does reveal a source of about 4×10^{-3} f.u. at the position of the supernova. The 2.8 cm observations, on the other hand, show no emission above 6×10^{-3} f.u. at the position of the supernova remnant.

Studies of the radio emission from this supernova are handicapped by its proximity to NGC5455, an HII region. But between December 1970 and December 1971 the emission from that part of the sky increased by 6×10^{-3} f.u. ; HII regions do not usually vary over that sort of timescale, and Goss *et al.* conclude that the variable radio emission may represent the first stages in the development of a radio emitting remnant.

Curiously, the observations reported now indicate a strong flux at 21 cm in February 1972, when Gottesman *et al.* (*Astrophys. J.*, **174**, 383 ; 1972) noted a disappearance of the source at 11 cm. Taking both observations at face value, this poses serious problems in defining a spectral index for the source.

As yet, it is too early for the observations of this young supernova remnant to provide convincing support for a unique theoretical model. The mechanism is probably non-thermal, but the radiation could be coming either from a region surrounded by the ejected material (perhaps even from a young central pulsar) or from outside the supernova shell and still produce the observed characteristics.

AGU

Geophysical Perennial

from our Geophysics Correspondent

TRYING to report adequately on the American Geophysical Union's annual meeting in Washington (April 16–20) is like trying to describe St Paul's Cathedral from a couple of picture postcards. An attendance of 2,500, with 1,200 contributions, makes it impossible to give anything other than a highly localized account, fortified somewhat by gossip.

This year there was a significant upturn (20%) in the number of papers presented. This appeared to be an across-the-board expansion, although obviously planetology, with several tens of papers on the Moon, Mars and the Allende Meteorite, profited particularly this year. Contributions on lunar geology were headed by one from Dr Harrison Schmitt, who was laconically described in the programme as "the first AGU member to visit the Moon". Dr Schmitt, of course, drew a big crowd but it was a matter of some disappointment that the other key speakers—Drs F. Gilbert (University of California San Diego) and C. Hosler (Pennsylvania State University)—had sparse audiences. Dr Gilbert's weighty approach to inverse problems, although not likely to be appreciated by the arm wavers, has an applicability well beyond the Earth structure problem to which it was first applied, and future geophysicists will ignore it at their peril. Dr Hosler's lucid account of experiments in weather modification deserved a bigger crowd not least for the many remarkable photographs he showed. It would be unfortunate if a trend were established away from such lectures. A not altogether desirable tendency seems to be emerging of the completely unstructured session in which several participants identified as working on the "Frontiers of Frontiers" talked (chatted might be a better word) about recent work with extensive interjection from the audience.

A most exciting development is the rapid advance being made in the discovery of criteria by which an earthquake may be predicted. Two distinct lines of research have proved fruitful. In the first, detailed seismicity maps of a region of the Earth are compiled and attention is paid to the aftershock activity from relatively large earthquakes. This probably delineates the region of slip. Frequently these aftershock zones do not overlap (at least in a substantial period of time) and this leads to the unsurprising conclusion that regions which have not recently been so delineated by aftershocks are most prone to rupture in the future. Drs J. Savino (Lamont-Doherty Observatory) and R. Wesson (US Geological Survey) examined seismicity as a tool for prediction in Alaska and California. Dr Savino found that the Sitka earthquake of 1972 occurred in an identifiably higher risk region. Dr Wesson demonstrated that in California, before moderately large earthquakes, the numbers of small earthquakes rise. Dr W. Ellsworth (US Geological Survey) bravely went as far as to predict, on the basis of the above criteria, a magnitude 4.5 earthquake at 36° 40′ N 121° 17′ E (near Hollister) within the next few months, and the evidence certainly seems plausible.

The second approach to seismic prediction depends on some remarkable changes in the physical properties of rocks in the vicinity of some thrust-type earthquakes. In the 1960s Russians observed some apparently premonitory signs of earthquakes in central Asia. Of these the most easily measurable was a change in seismic velocities of rocks in the vicinity of the fault. Dr Y. Aggarwal (Lamont–Doherty Observatory) reported the recent discovery of similar features before small earthquakes in New York State, and Dr J. Whitcomb (Caltech) described velocity anomalies before the San Fernando earthquake. Dr Whitcomb's results leave little doubt that it is the compressional wave velocity which changes—declining by about 20% and then returning to a normal value just before the quake. The region in which this effect occurred for the San Fernando earthquake seems to be particularly large, at least 50 km in linear dimensions. Dr C. Scholz (Lamont–Doherty Observatory) presented an attempt to unify many of the precursory phenomena now reported by Russians, Japan-

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Upper Mantle Viscosity by Stokes's Law

STOKES'S method for determining the viscosity of a fluid must be familiar to anybody who has been through even the most elementary of courses in experimental physics. In the classic version of the experiment, a small sphere is dropped through an effectively infinite volume of the fluid, and the viscosity is calculated from a simple formula involving the sphere's radius, density and terminal velocity. On the face of it, the method has little application to the Earth's mantle; but in a fascinating analysis in next Monday's *Nature Physical Science* (May 21), Christoffel and Calhaem show how the same simple principles may be used to derive the viscosity of the upper mantle.

The falling body in this case is a piece of the Earth's lithosphere. Barazangi *et al.* (*Nature*, **242**, 98; 1973) recently suggested, on the basis of an analysis of intermediate and deep earthquakes and the associated seismic attenuation, that beneath New Zealand a fragment of the descending lithosphere has broken off and has now reached a depth of about 600 km as it sinks into the mantle ahead of its parent body. From the thickness of the lithosphere beneath New Zealand and the vertical and horizontal distributions of the deep earthquake foci, Christoffel and Calhaem conclude that the sinking fragment may be fairly regarded as a sphere with a diameter of 60 km—a sphere because, whatever the fragment's original shape, its corners will by now have been eroded by frictional heating.

To determine the velocity of the lithospheric fragment it is necessary to know when it broke off and how far it has fallen since then. Assuming that such a break would have visible effects at the surface, Christoffel and Calhaem equate the time of the break to the changes in volcanic and tectonic activity which occurred in New Zealand 3.8 million years ago, at which time what is now the fragment would have been 380 km above its present position. This gives a velocity of 10 cm a year, assuming that the piece of lithosphere is falling under gravity and that there are no convection currents. The only other parameter required, the density of the fragment, was estimated by Adams (*New Zealand J. Geol. Geophys.*, **6**, 209; 1963) from surface gravity anomalies to be 0.05 g cm^{-3}.

Putting these figures into the Stokes's Law formula gives a viscosity value of 3.1×10^{20} poise, a constant value which represents the effective viscosity of the upper mantle over the depth range 250 - 600 km and over a period of almost 4 million years. If, however, frictional heating causes partial melting at the surface of the fragment as it falls, the diameter used to calculate the viscosity may be too high; and if the medium through which the fragment is falling is not effectively infinite, the measured terminal velocity will be too low. The Stokes's method viscosity should therefore be regarded as a maximum. Even so, it is in good agreement with Gordon's estimate (*Geophys. J.*, **14**, 33; 1967) of effective viscosity at a depth of about 600 km and only slightly less so with the estimate made by McKenzie (*Geophys. J.*, **14**, 397; 1967). On the other hand, it is significantly lower than upper mantle viscosity values derived from seismic behaviour and from isostatic readjustment. But as Christoffel and Calhaem point out, seismic and isostatic methods involve either complex models or time scales quite different from those of plate motions, and the resulting viscosity values thus have less relevance to plate tectonic theory.

ese and Americans in terms of the development of dilatancy in strained rocks, flow of fluids into the dilated region, the hardening of the rocks in dilated form and ultimately the release of strain by the rise of pore pressure along the fault. Both Drs Scholz and Whitcomb drew attention to a very striking correlation between duration of precursory phenomena and size of earthquake. If the correlation holds good in the light of further study it seems that a magnitude 8.0 earthquake would give tens of years of advance warning. In spite of the many questions this work raised during the meeting, particularly about the extent of its applicability, it is clear that earthquake prediction has seen a significant advance in the last year.

The problem of mantle hotspots underwent little resolution during the meeting in spite of many papers on the subject. Based on a suggestion by Professor J. Tuzo Wilson, Dr J. Morgan proposed three years ago that the long linear chains of volcanoes seen in many parts of the world are the consequence of the motion of plates over hotspots in the mantle. The fundamental questions seem to be four in number. (1) Do hotspots exist—that is, are there regions beneath plates where temperature is anomalously high? (2) Do hotspots have a permanence during geological periods of time? (3) Are they connected to any deeper expression of convection such as a rising plume of material? (4) Do they have a global influence on plate motions, that is, is a knowledge of hotspot characteristics necessary to an understanding of the driving forces of plates?

The surface evidence that "something is going on" is indisputable from the long island chains. It was very difficult, however, to perceive any compelling evidence from the many contributions that would cause the last three questions to be answered in the affirmative. There seems as yet to be no clear agreement on the definition of a hotspot (Morgan proposed ten; at this meeting it was suggested there might be hundreds). Evidence from the geological and geochronological record contains too many contradictions to be regarded as supporting wholeheartedly a positive answer to question (2). Very little was presented that bore on questions (3) and (4), but good papers were given by Dr J.-G. Schilling (University of Rhode Island) on the rare-earth geochemistry of ridges and plumes and by Dr B. Minster (Caltech) on the kinematic evidence for plate motions and consequent inferences on hotspot stability. It is, however, difficult to avoid the conclusion that the hotspot hypothesis is indeed no more than that and is in need of some stiff theoretical support as the observational material is so equivocal.

A sparsely attended session was devoted to the Earth Resources Technology Satellite. There can be more than one opinion of whether ERTS is yielding significant results but it was a disappointment that more did not come to learn for themselves whether ERTS has any uses for them. A particularly good technical introduction to ERTS was given by Dr S. Freden (NASA).

Perhaps the clearest impression with which one left the AGU meeting was one of vigorous controversy. There is disagreement about earthquake mechanisms, about plumes in the mantle, about the core-mantle boundary, about dilatancy in earthquake prediction and doubtless about all manner of other things.

METALLURGY

Superplastic Control

from a Correspondent

ONE topic of particular interest at present to physical metallurgists—grain boundary sliding—was the subject of a conference held at the University of Sheffield under the auspices of the Institute of Metallurgy on April 12. When a polycrystalline metal sample is deformed at relatively high temperatures, not only do the interiors of the individual crystals or grains deform (by well-understood mechanisms) to change shape consistent with the overall deformation but the grains also move bodily with respect to each other. The motion resembles the relative motion of peas channelled to pass through a funnel.

Dr D. McLean (National Physical Laboratory) opened the discussion at the conference by reviewing the evidence from elastic and anelastic damping measurements that grain boundary sliding results from motion of dislocations in the grain boundary in a manner similar to that in which lattice dislocations are responsible for plastic deformation of the grains. The situation is by no means clear cut, and different groups still have different concepts of the nature of dislocations in a grain boundary. This is surprising for, geometrically speaking, the position has been clearly defined by recent work of Dr W. Bollmann (Battelle Institute, Geneva). One indication of this lack of rigour in basic understanding is the proliferation of different nomenclatures for the differing concepts to accord with the various roles that the dislocations are expected to fulfil.

Some beautiful examples of dislocation arrangements in slid bicrystals of aluminium were shown by Dr C. A. P. Horton (Central Electricity Research Laboratories, Leatherhead). The electron microscope was again shown to be a vital metallurgical tool. It will surely lead to the definitive observations on the structural aspects of grain boundaries as it has already done for dislocation structures within the grains.

Grain boundary sliding is of great practical relevance in that the ease or not with which it occurs, relative to other mechanisms of deformation, controls the ductility of materials at high temperature. It is also intimately associated with the phenomenon of superplasticity (the occurrence of extended deformation before fracture). The difference in the behaviour of some commercial and non-commercial materials was illustrated in a presentation of some results on a 316 stainless steel by Dr R. S. Gates (Central Electricity Research Laboratories, Leatherhead) which revealed many unexpected features.

There is still controversy about how the most meaningful measurement of grain boundary sliding is to be made. Some sliding that does not contribute directly to the extension of the material may undoubtedly occur, whereas other sliding gives an independent contribution to the extension. It is only just being realized that the two contributions are evident in extreme forms in superplasticity and low temperature creep behaviour. It was clear from the animated discussion led by Dr G. B. Gibbs (Central Electricity Research Laboratories, Berkeley) that much thought and many more experimental observations on both commercial and non-commercial materials are needed before even certain conceptual problems of the sliding geometries can be finally straightened out.

It was of undoubted interest to metallurgists to learn during the course of the meeting that grain boundary sliding occurs in ceramic materials even though a superplastic ceramic is still in the realms of conjecture. Detailed information was provided by Dr P. E. Evans (University of Manchester Institute of Science and Technology) on alumina, zirconia and scandia and by Dr R. T. Pascoe (Central Electricity Research Laboratories, Leatherhead) on haematite iron oxide. In the field of ceramics the concept of dislocations in boundaries will have to be modified as the common structures are no longer preponderantly cubic as they are in metals. Furthermore, there are difficulties in interpreting both the activation energy and the stress dependence of the observed strain rates in these materials in terms of accepted mechanisms of creep deformation.

Clearly there is much still to strive for in this field. As yet a complete understanding of grain boundary sliding is some time away and much further work is required before the sliding characteristics of grain boundaries can be predicted and the desired improvement made in material properties.

SCIENCE IN EUROPE

Poor Outlook for European Collaboration

THE pages which follow provide a vivid illustration of the richness of European science but very little evidence that the integration of Europe in the economic sense will be accompanied by the joint effort in research and development which circumstances require. The underlying trouble is that European governments, disillusioned as they have become in recent years by the conviction that public expenditure on research and development is uneconomic, have not been able to grasp the notion that the long-term economic and cultural health of Europe depends as much on a vigorous scientific community as on any other ingredient of the common enterprise. And although the summit conference in Paris in October instructed the European Commission to prepare a plan for the integration of European science before the end of 1973, it becomes increasingly clear that the commission's own ideas of what should be done are at once over-ambitious and needlessly neglectful of existing institutions. If the precedents of the past few years are an accurate guide, the chances are small that the commission will be able to meet the December deadline. This is a serious cause of anxiety.

The arguments for a closer integration of European science are elementary and compelling. In academic research, however defined, there is an urgent need to remove the sense of isolation that afflicts a great many of those who work in European universities and research institutes. The American experience since the Second World War has shown quite clearly that there are advantages of scale to be exploited by the scientific community, but these derive as much from the mobility of people and ideas as from the scale on which money is expended. It is an enormous source of benefit that people should be able to congregate in groups with common interests, either permanently in departments or institutes dedicated to particular problems or in conferences and seminars. By comparison with the United States, Europe is not too badly endowed with active researchers, but people are much less free to move about. As time goes on, the way in which CERN has functioned as a focus of common activity in high energy physics emerges more and more clearly as an exception which proves the general rule that there are too few common European centres of intellectual activity in science. The European Molecular Biology Organization, it is true, may emerge as a similar institution, especially now that it will have a laboratory at Heidelberg to call its own, but that will take time. Organizations such as the European Space Research Organization, enmeshed as they have been in national rivalries and ambitious arguments for what is called a "European space policy", have conspicuously failed to function as common centres of intellectual enterprise. And it remains depressingly the case that in most fields of scientific work, European researchers are confined too narorwly to their universities and research institutions. The most conspicuous links between, say, immunologists

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in Holland or atmospheric physicists in Britain remain with the United States.

What the commission must recognize is that its first objective, so far as academic research is concerned, is to make arrangements for the greater mobility of people and ideas. To begin with, money is less necessary than the reorganization of national institutions—what the commission itself is fond of calling harmonization. Briefly, arrangements need to be made that will allow national universities and research institutes to employ Europeans from elsewhere within the community on equal terms. In the sense in which university researchers and teachers in France and Italy, for example, are civil servants, it is essential that in future other nations' citizens should become, if they wish, employees of the French and the Italian governments. In the long run, the best solution will entail some degree of disestablishment of the centrally controlled university system, but that is a hot potato. For the time being, the commission itself could usefully concentrate on some means by which the machinery of an equal employment policy could be created within the present framework. And it is not too soon for Dr Dahrendorf, the member of the commission responsible, to be asking how in the long run a framework should be laid for a European university system. Far better this than the commission's efforts, in the past few years, to define the rules for the transferability of professional qualifications from one country to another, now mercifully discredited by the simple argument that any such definition is bound to be at once needlessly restrictive and unfair to at least some members of the community.

The second need is that there should be some common European institution with money to spend on international conferences, seminars and travel on a European basis. In the past few years, national governments to their credit have been spending more generously in fields like this, but it has been left to national academies to handle what funds there are to spend, with the result that there has grown up a complicated system of bilateral agreements designed to ensure that each country can be confident that it derives as much benefit as it thinks it is entitled to. Valuable though this system has been, the time has now come when it should be abandoned and the best alternative is that the European Commission's own suggestion that there should be a European Science Foundation to administer funds like these should be implemented quickly. After all, if national governments are prepared, as seems likely, to contribute to a common fund for regional development, it is surely not unreasonable to ask that they should also make contributions to a much smaller fund to ease the movement of ideas and people from one place to another. It is entirely irrelevant that such a common fund would inevitably benefit the smaller European nations more immediately than the chief contributors. Indeed, if European collaboration means anything at all, it must surely follow that those most in need are most helped.

Another, more serious problem is the organization for spending money by way of grants on research projects of an academic character. By now, all the members of the European Community have well developed systems for spending money on research, but there are of course great variations from one country to another in the scale on which research money is made available. In Italy, for example, expenditure is by any standards low, while the criteria by means of which decisions are made leave much to be desired. Politically, it is unthinkable that there should be a speedy amalgamation of the research councils and their equivalents on a European basis, but there is at least a case for working out, even at this early stage, a framework within which there can be a common procedure for financing European research. By all accounts, the British view is that the relationships between British science and scientific research in other European countries outside the European Community are so important that national institutions cannot at this stage be subsumed in European institutions, but this is a false position. There is no reason why the community as a whole should not be concerned to foster links with other European countries as the British government and the Royal Society are supposed to be. Other European governments, the government of France, for example, are supposed to have responded to the commission's proposal for a European Science Foundation much more negatively, insisting that national governments must remain responsible for financing national research more or less indefinitely. But that, of course, is an argument that must be met head on. If the European Community means anything at all, it means that there must be a deliberate pooling of European resources. So it is essential to make an early start on some machinery for dispensing research grants on a European basis and the commission's concept of a European Science Foundation, muddled as it is, would be a good beginning.

Is there any convincing reason why the commission should not insist on this part of its proposal as an essential ingredient of the package of proposals it has promised by December?

On industrial research, there is much more muddle to contend with. The commission has traditionally taken the view that the biggest prize to be won is in the more efficient exploitation of industrial research and development, and there is a long and not particularly successful record of endeavour in this field. The European Launcher Development Organization, which has now, not before time, abandoned its attempt to make an efficient satellite launcher out of the relics of the Blue Streak rocket, is a splendid illustration of the kinds of machinery which should be avoided in the years ahead. The proposals of the Aigrain working groups in 1968 for common enterprises in the development of such machines as advanced computers and telecommunications equipment, essentially devices for pooling development resources within similar kinds of organizations, have luckily not come to much. And there has been very little applause for the commission's own ambitious scheme, put forward by Mr Spinelli a year ago, that would have turned the commission into a kind of supranational government with large funds to spend on industrial development schemes of various kinds. The trouble is that there can be no logical basis for the central sponsorship of industrial development which lacks a common framework for industrial operations as a whole. This, after all, is a field in which the policies of national governments are inseparable from their interests that national industries should prosper. It is flying in the face not merely of unreasonable chauvinism but of reasonable self-interest that nations should willingly support common research and development programmes in the expectation that the outcome will be an enhancement of the common good.

EMBO

Another Step Forward for the Laboratory

Last week the reality of a European Molecular Biology Laboratory in Heidelberg came closer with the signing in Geneva of the agreement to establish it.

Now that the agreement to set up a European Molecular Biology Laboratory at Heidelberg is signed and sealed, molecular biologists can look forward to the emergence during the next five years or so of a centre which should have a place in Europe similar to that held by the famous Cold Spring Harbor Laboratory in the United States.

Early Days

The idea of a laboratory on a European scale was first put forward a little more than ten years ago at a meeting at CERN —that shining example of European cooperation in science— where the signature ceremony took place last week. Later, in 1964, the European Molecular Biology Organization (EMBO) came into existence with the object not only of founding a laboratory but also of financing fellowships and summer courses. To begin with EMBO received $750,000 from the Volkswagen Foundation for a period of three years starting in 1966—the object was that the organization should prove itself and in the meantime seek more permanent support from European governments.

The first EMBO proposal for a laboratory (in 1968) envisaged something larger than the one that is now to be built. But the governments involved did not approve, to some extent at least because EMBO itself had not thought out in sufficient detail what the purposes of the laboratory were to be and what functions it was supposed to perform.

Then out of a meeting in Konstanz in 1969 came proposals that formed the basis of the plans for the laboratory that have finally won acceptance from ten European governments. It is intended that part of the function of the laboratory shall be to provide a service to national institutes in, for example, the development of advanced instrumentation. It will also carry out advanced training and will, like Cold Spring Harbor, be a centre for meetings both formal and informal.

Heidelberg

The site provided by Germany for the laboratory is deep in the forest at Heidelberg next to the Max-Planck Institut für Kernphysik, which together with the Deutsches Krebsforschungszentrum has offered temporary office and laboratory accommodation so that scientific work can begin quite soon, before the laboratory proper is completed.

As far as staff is concerned, the total number of personnel is expected to reach 130 in 1976 and the full complement of about 300 in 1978. Of this number nearly sixty will be qualified scientists and engineers (QSEs), including about sixteen postdoctoral fellows, and there will be about 167 technicians, fifty-five administrative staff and thirty to thirty-five visiting scientists (at any one time).

There are also plans afoot for establishing an outstation of the laboratory at the electron synchrotron (DESY) in Hamburg and one at the high flux reactor in Grenoble. This will make possible experiments with intense X-ray and neutron beams.

Expenditure

Table 1 shows the expenditure envisaged in the years until the laboratory is fully operational. It includes an estimate of the way in which a special contribution to capital costs made by the German government may be spent. Ratification by the participating governments is unlikely to take less than a year, but during that time sufficient money will be available for architects to be engaged and other aspects of advanced planning to be completed.

Table 1 Global Costs of the Laboratory to Contributing Governments

Estimated ceiling of non-recurrent costs	1973 May–December	1974 January–April	1974 May–December	1975	1976	1977	1978	1979	Totals
	Before ratification				After ratification				
Preliminary studies	71	63.5							134.5
Central laboratory			2,406	2,704	2,385	1,291	630	176	9,592
Outstation at DESY, Hamburg	568	140	213	77					998
Outstation at Grenoble	96	447	78	46					267
Total *A*	735	250.5	2,697	2,827	2,385	1,291	630	176	10,991.5
Possible application of German special contribution (DM 12,000,000)	537	113	1,100	950	579				3,279
Net non-recurrent costs *B*	198	137.5	1,597	1,877	1,806	1,291	630	176	7,712.5
Recurrent costs (estimated budget)									
Central laboratory			558	1,431	2,564	3,743			
Outstation at DESY, Hamburg	124	72	169	299	324	324			
Outstation at Grenoble	39	56	139	259	284	294			
Total recurrent costs *C*	163	128	866	1,989	3,172	4,361	5,066	5,595	
Global costs to governments ($B+C$)	361	265.5	2,463	3,866	4,978	5,652			

Costs are in thousands of accounting units at 1972 prices (1 AU=$1.2).

SPACE

European Unanimity Still Hard to Find

by our Special Correspondent

The future of the European Space Research Organization is well defined but the remainder of Europe's space programmes are still in a state of flux.

EUROPE is scarcely more united over space than it has ever been. The veneer of unanimity achieved at last December's meeting of ministers in Brussels at which plans for a European Space Agency were tentatively formulated, has already been disrupted by the sudden end of the European Launcher Development Organization's work late last month and an outspoken attack on British attitudes to European space efforts by Mr Charbonnel, the French Minister for Industrial and Scientific Development.

In a statement issued recently in Paris, Mr Charbonnel declared that at the Brussels conference only the French and the Germans had any real contribution to make. Britain's suggestion—sponsored by Mr Michael Heseltine, British Minister for Aerospace and Shipping — that a "European NASA" should be formed was all very well, Mr Charbonnel said, but if Britain is not prepared to put more money into either L3S, the French launcher proposal, or the post-Apollo work, "they are scarcely in a position to ask us, in the immediate future, to push ahead with the project of a European NASA. If the English are not interested in anything — scarcely in the post-Apollo programme, not at all in the launcher, then in the name of what can they ask us to create an integrated organization?"

Such a firm statement does not augur well for the ministerial meeting planned for July, when the French intend to ask the other European countries to guarantee to purchase and use L3S when it is built.

Space Agency

The future of the European Space Agency will also have to be sorted out against the background of ELDO's effective demise. The original plan was to merge ELDO with the European Space Research Organization if possible by January 1974. But the ending of the ELDO programme following the withdrawal of finance by the French and Germans means that in practice all there is to merge at present is ESRO's programme, a French launcher proposal that at present is only a proposal, and the various projects that are currently being nurtured in national programmes — for example Britain's Geostationery Technology Satellite.

It is well known that the agency, which is unlikely to be formed in anything but name by January 1, will have to be based around ESRO. Over the past five years the structure of ESRO, with its comparatively powerful secretariat which is largely left alone to get on with the job once the decision to proceed with programmes has been taken by member states, has proved effective. If ESRO is to be part of the new agency, then this authority must be preserved, and Europe must not destroy its one successful space venture merely to create an organization with an impressive sounding name.

But it is difficult to see how the agency can emerge with a really strong management structure if such projects as L3S are to be included. The French put forward L3S as an alternative to Europa III last year on the grounds that it would cost less and would be less risky. Mr Charbonnel has put the cost at about 2,400 million francs, and this figure is understood to include a 20% margin to allow for contingencies. The French proposal is that France will provide 60% of the finance and absorb any increases in the estimated cost of the rocket, providing that the rest of Europe, and in particular Germany, will contribute the remaining 40% of the finance.

But France is only prepared to do this if management control of the project lies in French hands. The French believe that the launcher can be built on time and at the estimated cost provided the management is strong. Yet at the same time, if the agency is created the French will doubtless want the launcher to have the stamp of European approval and cooperation, so that the agency is likely—if the French get their way—to have some sort of responsibility for the success of the project without having any real control over it. The agency might, for example, be expected to marshall the international finance for the project and keep an eye on progress so that it can report to the member states.

Similarly, Britain has offered its Geostationary Technology Satellite (GTS) as a collaborative project, but again control of the programme would be likely to lie within Britain which would provide most of the finance. This proposal by the British in fact clashes rather oddly with Mr Heseltine's insistence at the Brussels meeting that a strong European agency should be formed.

More Problems

Quite apart from these problems, there are enormous administrative problems involved in creating the agency. Integrating national programmes is one thing, but internationalizing national facilities and running expenditure is another. Countries with small national programmes are likely to have to increase their expenditure in order to pay for a share of the facilities that other countries already own, but which will be used for international programmes.

But if the structure of the new space agency is far from clear (and little progress has been made so far towards formulating it) at least ESRO's programmes within that structure are well defined.

Last month the organization chose two new scientific satellites, HELOS (Highly Eccentric Lunar Occultation Satellite) and ISEPS (International Sun–Earth Physics Programme) which will be launched in 1977 and 1979 (see *Nature*, **242**, 547 ; 1973).

In the nearer future on the scientific front COS-B, a cosmic-ray satellite, and GEOS, which will study particle fluxes and electric and magnetic fields, are scheduled to be launched in 1975 and 1976 respectively.

ESRO's programme also includes studies on Spacelab (part of the United States shuttle programme). Six of ESRO's nine member countries are now participating in the project (Italy, Spain, West Germany, Belgium, the Netherlands and Britain) and Europe has to tell NASA by August 15 if it intends actually to build the laboratory for delivery to NASA in 1979. It is possible that France also will contribute 10% of the cost to the project if West Germany comes in on the French launcher proposal.

Plans for Spacelab

At present, three possible concepts of the laboratory are being studied including a general purpose laboratory and one of a modular construction that might prove more flexible. The laboratory will be carried into space in the cargo bay of the shuttle and will provide working facilities for up to four scientists. Although, in terms of cost, the laboratory is a comparatively small part of the shuttle programme—the cost of developing, building and delivering the first example was originally put at about $275 million, while the cost of the shuttle itself will be thousands of millions of dollars — the laboratory is a key part of the programme.

Technically ESRO is fully committed to developing Spacelab, although the individual countries are not. There is, however, an escape clause, which states that if by August it seems as if the cost of the project will "unacceptably exceed" the original estimates, then Europe can withdraw from the programme. What "unacceptably exceeds" means has not been defined. But it seems more than likely that Europe will go ahead with the project, carried along by Germany's enthusiasm.

Later this year ESRO is to appoint a scientific mission definition group to help decide what experiments should be flown in Spacelab when it is launched in 1979.

Applications Satellites

The third prong of ESRO's current work, a particular growth area since 1971, is its applications satellites. Three programmes are currently underway. The European Communications Satellite programme provides for an orbital test satellite to be launched in 1976. At present competitive design studies are being made and a single contractor who will finalize the design and undertake development will be chosen by the end of the year.

The satellite will carry 5,000 telephone circuits and will be inserted into the European telecommunication system so that the telephone undertakings can see for themselves that it works. An operational satellite with a planned life of seven years should be launched in 1980.

The other applications programmes are Meteosat and Aerosat. Meteosat will take visible and infrared pictures of the Earth with a 40 cm telescope once every thirty minutes froma geostationary position over Africa. The satellite will play two roles—first, as an experimental satellite in the international Global Atmospheric Research Programme, and then as an operational tool in the World Weather Watch which is to follow the large research programme into weather formation that is planned for 1977.

Aerosat, at present the most controversial of ESRO's applications satellites, is a joint programme with the United States with Australia and possibly Canada and Japan participating. ESRO is now selecting a United States industrial partner to co-finance the development of the satellites. Comsat, ITT, Fairchild, RCA Globcom and Western Union are the companies who have applied to join ESRO in the enterprise which will put two satellites into orbit over the Atlantic in 1977. The final choice of industrial partner should be made next month.

The project is an ambitious one intended to evaluate the possibilities of communicating with hundreds of aircraft at once and of running surveillance of them by satellite so that air traffic control over the oceans can become more precise.

Finally ESRO's programme also includes paper studies of second generation applications satellites—a maritime satellite (MARSAT) to assist navigation, an Earth resources satellite, and a television broadcasting satellite. All these are at a very early stage where ESRO is talking to the potential users of the systems in a process of mutual education in which ESRO learns what is wanted while the users learn what can be done, MARSAT is the most prominent of these programmes, as Britain's GTS is also a maritime satellite, and Britain's offer of it as a cooperative project has led to a comparison of the two satellites by ESRO. The results of this study should be available shortly, and it is by no means certain that GTS—which is essentially a technology satellite—will prove a suitable base on which to build an operational satellite.

Increasing Confidence

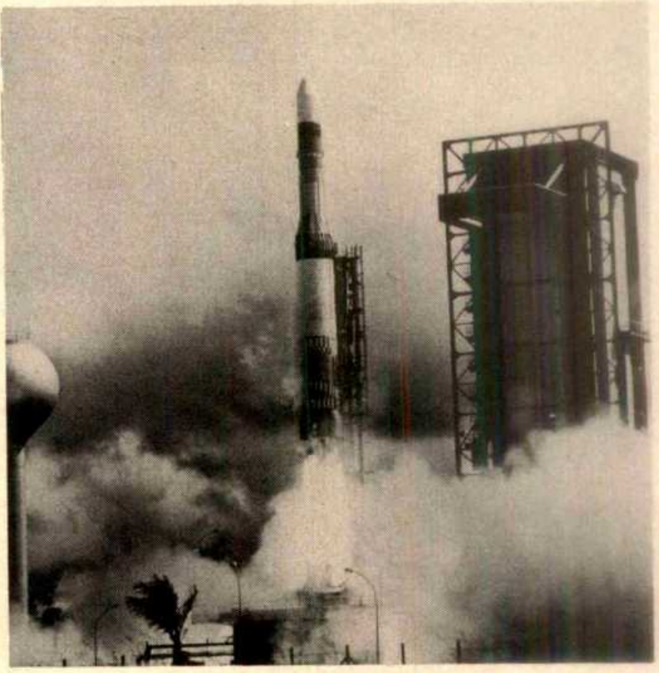
This extremely solid position has grown from the uncertain days of 1970 when expenditure was less than half what it is today, and when France and Denmark were considering withdrawing from the organization because the member countries could not agree on an overall programme.

ESRO's past record is impressive. Since 1964 seven scientific satellites have been launched, of which five have been completely successful. ESRO-1b, launched in 1969, was something of a disappointment, although it did collect some valuable data, and TD-1A, Europe's largest and most expensive satellite so far, suffered badly from the failure of its two tape recorders. Nevertheless faced with the problem of collecting data in real time, ESRO has put together enough tracking stations round the world to collect about 80% of the data from TD-1A.

Of the seven satellites, the last six have all been delivered on time to NASA for launch, and since 1967, when the cost estimates for TD-1 and TD-2 went off beam, ESRO has built all its satellites to cost. The willingness with which NASA is prepared to cooperate with ESRO on the post-Apollo studies, and the way in which five United States industrial companies are competing to put their money into a joint programme with ESRO on Aerosat, is a mark of ESRO's standing outside Europe.

Downhill

The repeated failures of ELDO, however, are quite another story. Founded in 1964 following pressure from Mr Peter Thorneycroft, then Britain's Minister for Aviation, who wanted a continued use for Blue Streak, the original estimates for the development of a launcher was about $195 million to be spent over five years. In the event, ELDO has spent $720 million over nine years, all to no effect. Eleven launches have resulted in five successes (the first five launches) and six more or less disastrous failures. Britain's Blue Streak functioned perfectly each time — the first launches were either Blue Streak alone or Blue Streak with dummy top stages —



First and last launch of Europa II.

but repeated failures in stage separation and ignition of upper stages coupled with rising costs gradually sapped confidence and Britain's decision to withdraw in 1968 was in many ways the beginning of the end for ELDO.

The mistakes made in forming ELDO and running it are well known. The attempt to strap together assorted bits of European rocketry to make one powerful launcher was at best ill-advised, but much more damaging was the attitude of the member states that formed ELDO. The organization was set up in such a way that it had little control over the activities of the companies that built the rockets, and the way in which the programme was endlessly strung out was guaranteed to breed political crises. Several failures in a short length of time would have been far less damaging and far more acceptable.

The failure of ELDO undeniably lies with the member states rather than the organization's secretariat. Where Europe has had the courage of its convictions—in providing ESRO with a strong structure—it has had valuable returns for its money, and if the new agency is to succeed then it too must be given the freedom it needs to carry out programmes, and must not just be given a coordinating role.

EUROPEAN ECONOMIC COMMUNITY

Science Policy in the Balance

by our Special Correspondent

The past few months have been a critical time for science policy within the Community. Commissioner Spinelli's policy for science has not found favour with the European countries and a reappraisal of science policy is currently being carried out. In this article the difficulties surrounding the making of a science policy for the Community are analysed.

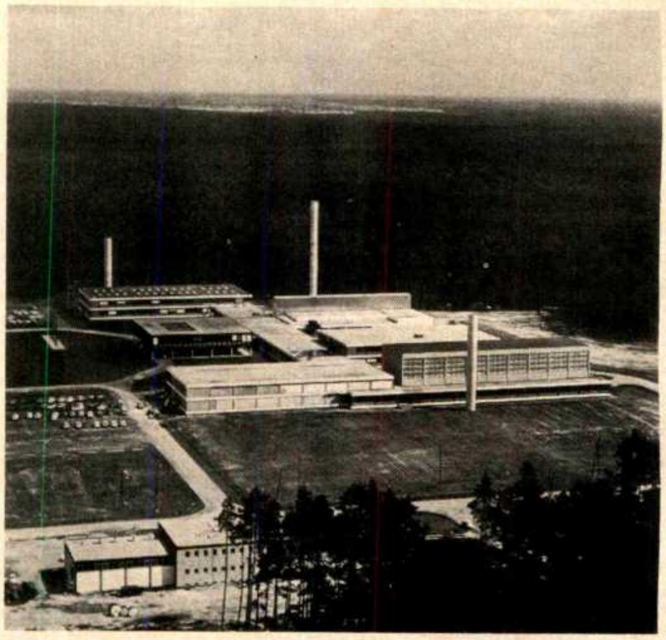
Commissioner Altiero Spinelli's carefully constructed plan for a common policy for scientific research and technical development within the European Economic Community lies in shreds after being considered by a special committee set up by the permanent representatives of the European countries in Brussels.

This setback places the Commission in the embarrassing position of having to produce an acceptable science policy by January 1, 1974, as ordained in the summit meeting at Paris in October 1972. At best this will be a race against time now that a completely new outlook on science within the community is necessary, and at worst the community will have to make do without a science policy until well into 1974.

But what has caused the downfall of the Spinelli document which was launched with such enthusiasm? In June 1972, Mr Christopher Layton, at that time Spinelli's chef de cabinet, came to London to publicise the contents of the document, but it seems that within the British Government, in particular, the document was met with indifference. From all accounts the French also objected to parts of the policy document, and with some support from other countries the British and the French between them have succeeded in completely deadlocking the Recherche Scientifique et Technique Committee set up by the Council of Ministers which has been examining the report. On May 8 the science ministers of the Community were supposed to meet in Brussels to discuss the Spinelli document but when it became clear that no agreement could be reached the meeting was postponed.

In essence, the difficulty with the Spinelli document is that it calls for the individual nations to lose too much of their autonomy too soon. As an example the third resolution of the plan called for "the Community to be informed of all plans and programmes which involve financial aid from public funds and because of their scale or type may be of Community interest, and to these ends take all necessary steps to lay down the criteria for this information and ensure that it is received in time". Clearly this was too much even for a country newly committed to Europe to swallow.

But another serious difficulty with this plan, which has been highlighted in recent months, concerns the call for a European Science Foundation to be set up. On this particular issue the feeling among delegates in Brussels and scientists in Europe has been that such a foundation, if set up, should not be restricted to countries within the Community and that other European countries should be invited to become members. Meetings have been held, first at the Royal Society in Britain last December and more recently in Munich by representatives of research councils and



The Joint Research Centre's establishment at Karlsruhe. The chief activity at the centre is the study of plutonium-containing nuclear fuels.

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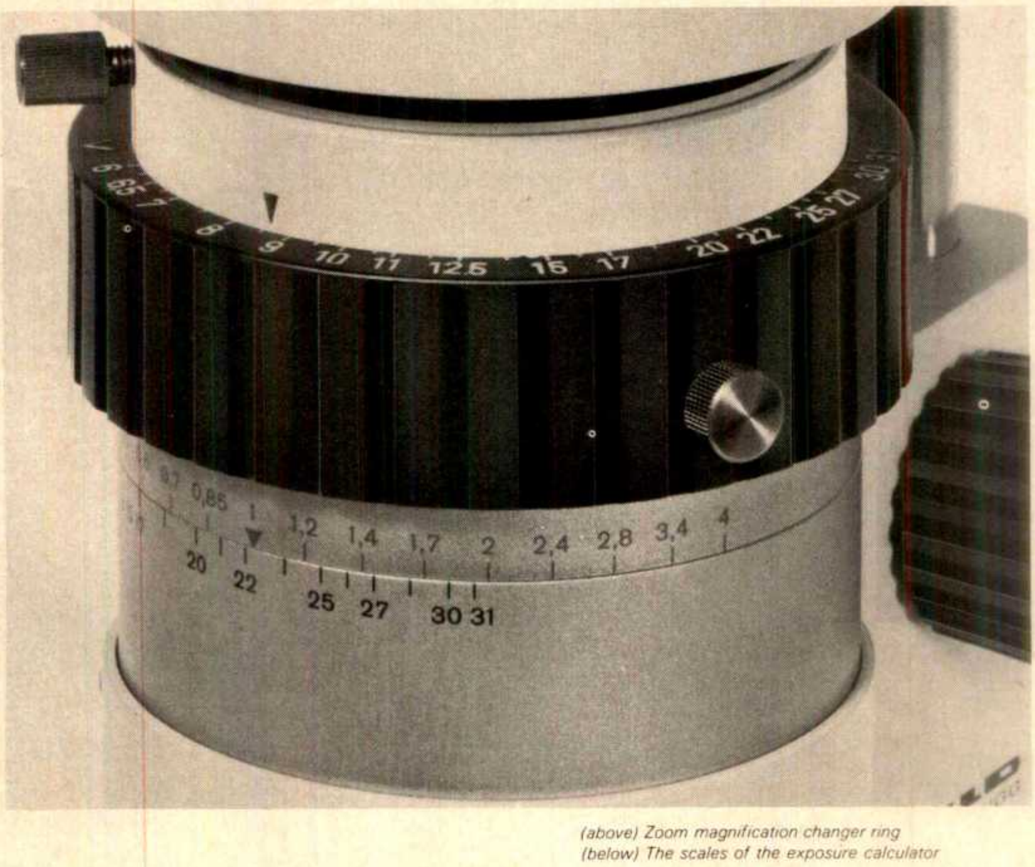
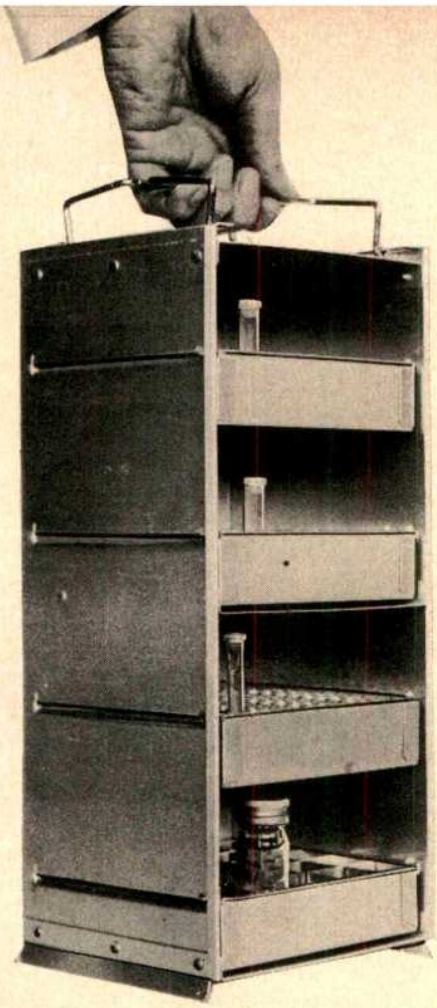


(above) Zoom magnification changer ring
(below) The scales of the exposure calculator



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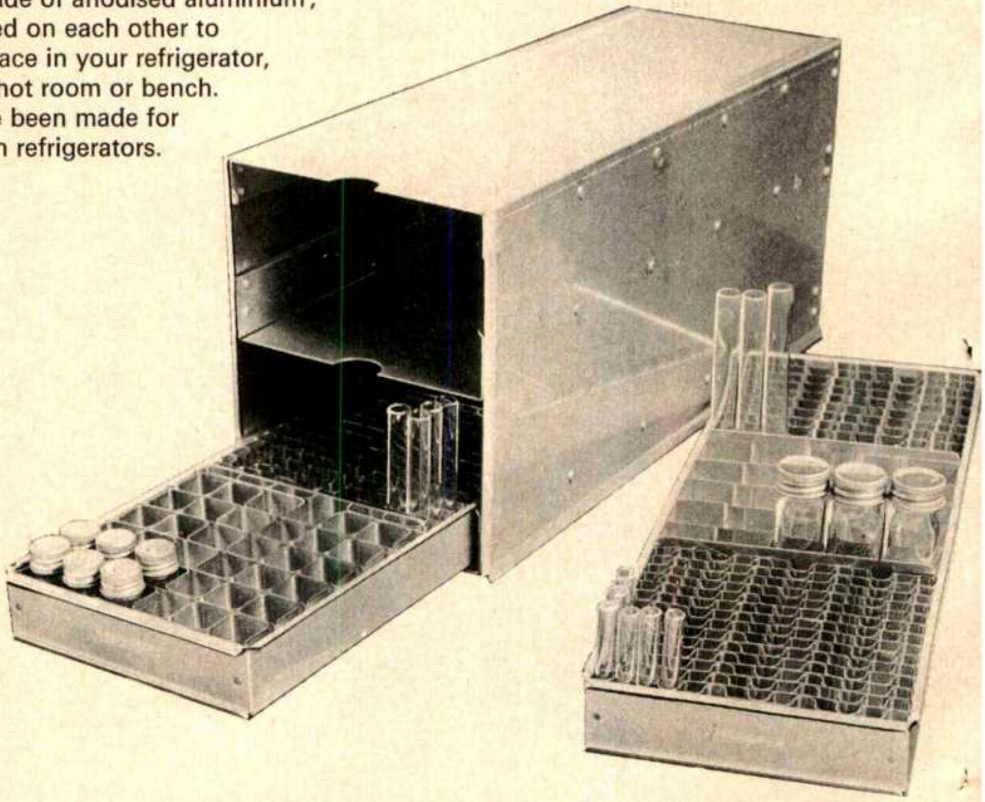

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academies of Europe to determine whether or not a common feeling exists for the setting up of such a foundation. A further meeting will be held in Paris in September at which it is likely that some resolution will emerge favouring a Europe-wide science foundation.

So even if detailed agreement with the Spinelli document has not been obtained among the representatives of the nine member states it is likely that the scientific community within Europe will take matters into its own hands and produce a proposition which the Commission will be asked to support.

Another item in the Spinelli plan, which has come to fruition in the past few months, is the recommendation that an advisory body be set up akin to the Advisory Board of the Research Councils in Britain, composed of independent and non-official representatives, who would be charged with advising the Commission on research and development matters. This committee, called the European Committee on Research and Development (CERD), has now met once under the chairmanship of Professor Casimir of the Netherlands. Mr Spinelli also recommended that the Commission should have a full-time science adviser appointed on a two-year renewable contract who would also act as chairman of CERD. As yet no decision has been taken to appoint such a person.

But in spite of the implementation of these specific parts of the proposed common policy on science and technology the chief part of the document lies dormant. So how is the Commission going to be able to produce an acceptable science policy by the end of the year? As luck has it, responsibility for science and technology within the Commission now rests with the German, Professor R. Dahrendorf. Professor Dahrendorf is taking a completely different line towards science policy, at least for the time being, to that taken by his predecessor. The new policy which is at present being drafted, and is unlikely to see the light of day outside Brussels until well into the autumn is, from all accounts, being framed in terms of specific projects for international collaboration rather than being based on broad philosophy as was the Spinelli document. Clearly such a document is much less likely to run into trouble with the science ministers, and if the collaborative projects are chosen sufficiently carefully then what will pass for a science policy will become accepted by the member states not unduly late next year.

In the light of the experience of the past few months the policy document prepared by Commissioner Spinelli and his advisors has been shown to be premature. Without doubt, a more cautious and conservative approach is needed when drawing up Community-wide policy statements, at least at this stage in the development of the Community.

The past few months have seen the emergence of CERD from being a dream of Spinelli's to being a reality. But the question which is being asked in Brussels and elsewhere is what will be the relationship of this body to the COST and PREST groups. PREST is a committee set up in 1965 by the Community's Medium Term Economic Policy Committee as a committee on scientific and technical research policy to "study problems which would be posed by the formulation of a coordinated or common policy for scientific and technical research and to propose measures to enable such a policy to be put in hand bearing in mind such possibilities as may exist for cooperation with other countries". When it became clear that such international programmes within Europe needed the cooperation of countries outside the then community of six countries COST was set up to include 19 countries—all the European non-communist countries. The work of COST was highlighted in last year's European supplement of *Nature* (**237**, 202; 1972).

But is there a need for both PREST and CERD, and if there is, what is the relationship between the two? First, CERD is composed of individual experts to advise the Commission while PREST is a body composed of official representatives from the respective countries which therefore is more closely allied to the Council of Ministers. But it is indeed possible that some conflict could arise between the two bodies.

As an example it seems likely that when Professor Dahrendorf has drafted his new science policy for the community he will ask CERD to comment on it. It will then be passed on to the Council of Ministers, which could either set up a special committee to study the document, as it did for the Spinelli policy document, or it could call upon the PREST group consisting now of representatives of the nine member countries to study the document. The feeling in Brussels is that the Council will adopt this latter course for the simple reason that the Dahrendorf policy document is expected to contain several recommendations for international research projects which is just the field of interest of PREST. Clearly the document will not be passed on to the Council until CERD approves of it. But what if PREST takes exception to the document or to parts of it? Such a situation will lead inevitably to an unhealthy conflict, the mere thought of which causes cold shivers to be evident in the Commission building in Brussels.

But is there a solution to all this? At present there is no link between CERD and the PREST and COST groups, but it would not be a surprise to some people if the science adviser to the Commission—if he survives the Dahrendorf redrafting of the policy—will turn out to be someone who is also intimately connected with PREST and COST.

This past year has seen a revival of the fortunes of the Joint Research Centre of the European Community. For the first time since 1968 the four laboratories of the centre have a four-year budget to be compared with the one-year contracts on which they have been operating in recent years.

The four-year budget, which was agreed on February 6 this year, is for 180 million units of account (one unit of account was one dollar before the recent devaluation). This will provide employment for 1,700 scientists and support staff.

Most of this—157.2 million units—will be spent at the Joint Research Centre which will employ 1,440 personnel.

Table 1 Projects to be Carried Out by the Joint Research Centre

	Appropriation (million units of account)		People employed
	1973	Four years	
(*a*) Nuclear projects			
Waste treatment and storage	1.57	6.90	75
Plutonium and transplutonium elements *	4.95	13.00 8.65	210
Materials science	1.94	8.50	89
Reactor safety	4.83	21.10	232
Applied data-processing	1.38	6.05	51
Information analysis bureaux	1.16	5.10	51
Central Bureau for Nuclear Measurements (CBNM)	4.45	20.35	170
Technical back-up for nuclear power plant operators	1.40	6.10	60
Training	0.33	1.45	15
Surveillance/management of nuclear materials †	1.23	5.40	57
Contract research	0.42	1.85	17
Management and coordination	1.80	8.10	79
(*b*) Non-nuclear projects			
Standards and reference substances	1.24	5.40	62
Environmental protection	2.97	13.00	142
Remote sensing of Earth resources	0.24	1.05	10
(*c*) Petten high-flux reactor ‡	5.26	23.00	95
(*d*) Use of Ispra-1 reactor	0.50	2.20	25

* Supplementary programme involving all countries except Italy.

† Supplementary programme involving all countries except France.

‡ Supplementary programme involving Germany, Belgium and the Netherlands.

Although the research programme is for four years it will be reviewed in two years' time. The detailed programme is shown in Table 1.

Since the ministers agreed on the programme of research outlined in Table 1 a further 20 million units of account have been allocated to the Joint Research Centre to be spent over the next four years. The chief item of this list is a sum approaching 2 million units of account to be spent on producing hydrogen by nuclear power.

But one of the most cheerful notes to be sounded about the community's research plans in the past year is the renewed interest in the possibility of obtaining power from a controlled fusion reactor. Between 1972 and 1976 the Commission will spend 56,196 million units of account on fusion research, all of it on contract to laboratories within the enlarged community. This sum represents only about 30 per cent of the total being spent on fusion research, that being the percentage aid granted by the Community.

But there is great enthusiasm in Brussels about the Community's fusion programme. There is a feeling that here, at last, is a programme that will achieve all that Euratom set out to do when it was set up in 1957 but in the field of nuclear fission. The nuclear fission and reactor part of the Euratom programme ceased to be viable at a very early stage when no agreement could be reached on what type of reactor to develop in the Community. The vital question was whether the Community should concentrate its efforts on gas cooled or water cooled reactors. The unfortunate fact at the time was that each individual country had developed its own research to such a stage when Euratom was set up that it was embarrassing for them to have to sacrifice so much knowledge to contribute to a Community programme. The same will not happen in fusion, it is argued, for the research is still at an early enough stage and the expense of the research is such that no single country within Europe can support research in all aspects of nuclear fusion. It can only be hoped that the cautious optimism which surrounds the fusion research programme will prove to be well founded.

What is the long term future of the Joint Research Centre? No longer are these laboratories solely geared to nuclear projects as can be seen from Table 1. The question is whether Commissioner Dahrendorf will include some comment about the future of the Joint Research Centre in his policy document. Little contract research is done at these establishments at present. But maybe this is where the future should lie. The Harwell establishment of the United Kingdom Atomic Energy Authority has proved its worth in recent years by going more and more into contract research and in fact earning a great deal of money to offset against its budget. Perhaps the Commission should be thinking along such lines for its own laboratories.

COMPUTERS

Need for Collaboration in Europe

M. ALLEGRE

120 Rue Cherche du Midi, Paris

Computing points the way which must be followed by other industries before true European collaboration can be realized. Mr Maurice Allegre, who has been closely involved with France's "Plan Calcul" indicates in this article the way in which a new progressive change in attitude can lead to the effectiveness necessary in such a competitive sphere.

THE young computing industry is original in many respects. Within twenty years, computing has become one of the major world industries, having a large effect on daily life. Further, the leading computer country is undoubtedly the United States, and its example is unrivalled in the economic history of the world.

After two years of uncertainty, this industry has just begun to move forward again, admittedly at a somewhat more sober pace than before, but nevertheless sufficiently rapidly to guarantee a rosy future. For many years, IBM, with more than 50% of the world market, has held the record for capital investment; projecting current trends one might easily predict that, by 1985, IBM could also become the top company in the world as regards turnover, even overtaking General Motors. In the United States, however, as in the rest of the word, certain reactions are setting in which might modify this prediction.

Situation in United States

First, the government in the United States is seriously concerned about the disadvantages of this dominant position. Moreover, these doubts are not new, because with the exceptions of the United Kingdom and Japan, where longstanding protectionist policy towards national industries exists, it is in the United States that the role of IBM in equipping government departments has been most severely reduced. Today, the action of the United States government has become more incisive; after several minor skirmishes in the past, the whole weight of the antitrust laws is now in force and, for the first time, the openly affirmed intention is nothing less than to "dismantle" the computer giant completely. The existing world organization of IBM, however, makes this plan somewhat difficult to put into operation. Certain more limited steps, such as the obligation to publish well in advance and in great detail any departures from normal techniques will, however, allow the more serious effects of this domination to be avoided. This is a real danger, as IBM seems to have understood from the way in which it has just compensated Control Data in an out of court settlement of an antitrust case.

The IBM case will perhaps one day be as famous as the Standard Oil case, Standard Oil, of course, was divided on a regional basis, because of its monopolistic domination of the market—the classic example of antitrust legislation at work—giving birth to such offspring as Standard Oil

(Ohio); in the same way, IBM might be divided, with IBM (Europe) forced to become a separate entity. Another possibility, however, is division by conduct, a prospect which is perhaps even less appealing to IBM than regional dismemberment. One thing is certain: among IBM's American competitors, the list of failures has not ended with General Electric and RCA and the sole hope of survival in face of the renewed aggression of IBM lies in the antitrust action of the United States government.

Relevance to Europe

Things are also beginning to happen outside the United States. Foreign domination in such an important field has within the past decade led to reactions most aptly termed as "economic nationalism". Though humble in origin, these seeds are today beginning to bear fruit, and they will have a considerable effect on the international scene. More than six year ago, France launched the "Plan Calcul" for reasons which have been justified by the passage of time. Germany quickly followed suit, and Britain has for a long time shown a great deal of concern for its own computing industry. Japan has long since been past master of the art of simultaneously promoting and protecting a national industry and has created a computer industry on a large scale, and other countries, notably in Europe, are today on the threshold of similar projects. At this point one must also stress the great efforts being made in Eastern Europe, where the Soviet Union and other countries have generally been sharing out the tasks, undertaking to produce a range of compatible computers. I have recently returned from Moscow where I had the privilege of being present at a general presentation of these machines. The technique is not yet very elaborate; but its existence is proof of the depth and continuity of the effort made by these countries, for the same reasons as the Western world.

The Future

During the next few years, Europe will play an increasingly important role in the competition which has only just got under way. With a more rapid growth rate than the United States, Europe should, by the end of the present decade, have something like 30% of the world market, while the United States will have barely 50%. This illustrates the importance of the advance on the old Europe, which at present has only some 25% of the market.

Europe has for some time been aware of the problem in the computing industry. For example, since 1968, I have been president of a specialist computing group in Brussels within PREST with responsibility for putting forward a "harmonization" of scientific and technological policies among member states of the European Community, and I have come up with an interesting observation. All the members spontaneously agreed that computing was first and foremost an industrial matter and that one has to make the distinction between European branches of organizations whose decision-making bodies lies outside the European Community and community-based companies. A common problem is that of the necessity for the development of a truly European industry. Undoubtedly the idea of a powerful computer produced in common by all the European manufacturers came too soon. Nevertheless, the ideas have been planted and will be brought to fruition sooner or later in one form or another—they are already in the process of being realized. A report at present in preparation by the European Commission will soon give them official blessing.

Three European countries have already taken the decision to help the development of their national industries. But although their intervention may seem unusual, it is intended to redress the balance of opportunities and to make possible competition which would otherwise have been futile. The facts bear out this attitude, just as heavy international competition today forces the pooling of individual effort. Aware of the "economies of scale," European governments now encourage their industries to merge. Since the launching of the "Plan Calcul", France has stressed that this does not mean a simple operation at national level. On the contrary, it means the implementation of a programme in two stages which can be summed up thus: "Exister d'abord, coopérer ensuite".

Already many links have been forged between advisory and service bodies in French computing, where this sector is highly developed, and English software manufacturers. Recently in a sector which might be termed computer peripherals, and which regroups the manufacturers of minicomputers, terminals and peripheral equipment, an agreement has just been signed between a French and a German mini-computer manufacturer. The movement towards restructuring is only just beginning to develop in Europe. But the computing industry is above all about computing systems. It is better to use that expression than that of computer manufacturers which is today far too narrow. Having given proof of its existence by putting on the market an original range of medium and large computers, the Societe Française Compagnie International pour l'Informatique (CII) has for the past two years been able to move on to the second stage of the programme—cooperation. After many months of discussion, no agreement was reached with ICL because the British seemed more than anything to want to retain certain technical peculiarities—it is not easy to construct a common car when, completely against common practice, one partner wants to put the driving wheel on the right-hand side. On the other hand, the withdrawal of RCA allowed Siemens, which had taken over RCA's licence, to sign a general agreement with CII in February 1972 as a result of which the two firms decided to share all the tasks of planning, manufacturing and marketing computing systems. The Dutch firm Philips is at an advanced stage of negotiation to join the other two companies, and the past year has seen a new task, as yet unnoticed, get under way—that of the precise definition and identification of the future CII and Siemens range. Further, since January 1, 1973, the Siemens group in France has been totally integrated into CII and a reciprocal agreement operates for CII in Germany.

With a remarkable spirit of independence, the German firm Nixdorf is for its part trying to resolve its own dilemma: to get out of the tight computing stranglehold, in which it has succeeded so well until now, and so come to some necessary international arrangements. One may well fear an eventual Nixdorf-ICL agreement which would ultimately lessen the chances of a more general but nevertheless more desirable regrouping with Europe.

As for ICL, will it be able to resist certain temptations from the other side of the Atlantic? The United Kingdom government seems to be heavily orienting its national protege into international cooperation, primarily in Europe. Perhaps it would be advisable to start with an agreement on certain peripheral matters. In any case, in the long run there will only be room for one large truly European computing organization, and the way in which ICL will eventually come to join the already formed continental nucleus will perhaps become clear in due course. The three ministers concerned in France, Germany and the United Kingdom clearly expressed their wishes in this direction to their national companies at the end of last year.

Finally, as they awaken to the computing possibilities, other European countries should come to join the multinational body now being constituted. When it has been sufficiently organized, this body, backed by a large share of the European market, can think about opening up the American market, for example by a balanced cooperation with one of the small fry remaining on the other side of the Atlantic. Until such time, any cooperation with an American company can only result in imbalance in favour of one or other of the partners. It is certainly unthinkable that the European companies and governments should even unobtrusively abandon their many past efforts. On the other hand, a more rapid development in the American situation could create favourable opportunities for European firms. They must not come too soon, however, if we are to be able to take full advantage of them. One last point is worth mentioning: in Eastern Europe as in the West, the process of unification is in progress and the same causes—the necessity for a computing industry, and the desirability of a joint approach—are having the same affects. Will these two European industries remain forever on parallel tracks? Without envisaging events which cannot happen because of economic and political differences, it is nevertheless probable that certain points in common should be found in the respective interests of the two parties.

Having thus gained a right to exist through a minor share of the market by sheer strength and active government aid, the European computing industry is beginning to increase its capacity for future competition by creating a truly multinational unit, that is a unit in which there is no decision-making body with superior powers to any other. In order for a united Europe to exist, there must first of all be collaboration on an industrial level. To achieve this, European manufacturers are committed to achieving a new method of cooperation which will give real meaning to the concept or multinationalism. The risk is considerable but it is worth taking. In this sphere, computing seems destined to set an example in the difficult path it has embarked on.

Faith in the importance of the task and in the necessity for it to succeed breeds the desire to innovate and seek new formulae. From these will come a new progressive change in attitude which alone can result in effectiveness which is vital in such a highly competitive sphere.

IRELAND

New Moves in Irish Science

C. Ó hEOCHA

University College, Galway

The chairman of the Irish National Science Council describes the organization of research and development in a small country with a modest science budget.

Boyle, Kirwan, Higgins, MacNeven, Rowan Hamilton, Kane, Stokes, Tyndall, Fitzgerald: a formidable, but partial, list of great Irish-born scientists[1]. Technologists and inventors could be added, such as Muspratt, Holland and Parsons, as could Bernal, a pioneer of the concept of science policy. In the words of an editorial in the *Endeavour* on Irish scientists "the quality of their work is noteworthy for so small a country and deserves wider appreciation"[1,2]. Science in Ireland has for long been fostered by the Royal Dublin Society (established in 1731) and the Royal Irish Academy (1785), and schools of science, engineering and medicine have long histories in the university colleges. Nevertheless, conditions in Ireland were such that many of its most distinguished scientists performed their work outside the country.

A little over fifty years ago, twenty-six counties of Ireland gained political independence and successive native governments have assisted industrialization. But political problems, both internal and external to the country, caused emphasis to be placed for many years on industries catering for the home market. When national economic and social planning was introduced about fifteen years ago, it helped to bring about a period of sustained economic growth, and the industrial and service sectors, in particular, expanded and export trade developed greatly.

Since independence, scientists and others have worked to build up indigenous science and technology of international standard, and since the late 1950s the scale of scientific activity has developed rapidly, particularly in disciplines related to agriculture. Thus, when the British Association for the Advancement of Science met in Dublin in 1957, a listing of scientific institutions did not contain An Foras Talúntais (The Agricultural Institute)[3]. This institute was, in fact, established in the following year under the dynamic directorship of Dr Tom Walsh, and it is a measure of his success that by 1967 it accounted for 59% of expenditure on research and development within the government sector. Facilities for education and technical training were also improved during this period—universities expanded and new technical colleges were established. Economic growth was given an impetus by the creation in 1966 of the Anglo–Irish Free Trade Area and, most importantly, by the adherence of Ireland to the European Economic Community in January of this year. Membership of the community, however, creates problems as well as opportunities for a small country such as Ireland. The improved contact with neighbouring countries will be stimulating, but membership highlights the need to concentrate in our scientific as well as other activities on those areas which give the country comparative advantages.

Resources

Resources are, of course, limited—the population is only 3 million and it is unbalanced in that there is a preponderance of young and old people as a result of sustained and heavy emigration, which fortunately has slowed down in recent years. On the basis of gross national product (GNP) per head, Ireland is the least developed member of the EEC[4]. Expenditure per head on research and development, and the ratio of gross expenditure on research and development (GERD) to GNP are also lower than in the other member countries. On the other hand, if the number of papers published is any criterion, the productivity of Irish scientists is high. Thus, when the number of published papers listed in the five editions of *Current Contents* in 1971 is related to the populations of the countries of their origin, Ireland is seen to be on a par with Austria, Belgium and Finland, and not far behind the Netherlands, France and Norway. As pointed out at a FEBS meeting in Dublin last month by Dr A. H. Hughes, however, "while publication is the measure of academic progress, in the brutal world of business the better your work the less likely it is to be published". Man does not live on science alone, no matter how acceptable to international journals.

That there are structural weaknesses in the science system in Ireland is apparent from the research and development statistics[5,6] (figures for the science budget as a whole are not yet available in the detail needed to base the analysis on them). The total manpower involved in research and development in 1969 was 3,850, of whom 1,550 were graduates and 1,000 technicians. The overall expenditure on research and development is low (£14.3 million, or 0.74% of GNP, in 1971, of which the government supplied 60%); it is expended in the following sectors: government, 43.4%; business enterprise, 39.7%; higher education, 14.2%; private non-profit, 2.7%. Most of the expenditure is aimed at improving the country's economic capability, with considerably lesser sums being devoted to community services and the advancement of science; there is no expenditure on defence research and development. Between 1967 and 1971 support for research and development has grown at a rate of more than 12% a year in real terms.

Government Sector

Within the government sector there are only a small number of bodies concerned with research and development, but they use a very high proportion of research funds from public sources. Although the absence of a multiplicity of institutes is fortunate, the failure by other sectors, notably industry, to develop the capability to attract public funds, particularly for experimental development, implies a weakness which contrasts with the situation in many developed countries. Industry in Ireland consists largely of small firms having limited scientific expertise and thus having difficulty in assimilating relevant technical information, let alone performing research or development. There are, however, recent signs of improvement; for example, expenditure on research and development in the business enterprise sector has been rising steadily, even though such expenditure is small in relation to industrial output and is mostly confined to a very limited number of firms. The industries which devote most funds to research and development have not changed appreciably for some years: the food and drink group is by far the largest spender, followed by the electrical and electronic, utilities, and chemicals and drugs groups. Expenditure on research and development in science-intensive industries is still low and has not kept pace with the growth in exports from these industries; this is a reflexion of the growth of investment from abroad in export-oriented science-intensive industries[7]. To date such Irish-based subsidiaries have established few research and development facilities in the country. Efforts to attract such facilities are being intensified and a research park at Naas, sponsored by the Industrial Development Authority, has had some success. The encouragement of technological innovation in firms committed to grow in Ireland is being encouraged, with skill-intensive industries particularly favoured.

These considerations highlight the important role of the government-supported Institute for Industrial Research and Standards, which accounts for most government research and development funds for industrial research (about 16% of the 1971 total and 14.2% of that in 1969).

In fact, government expenditure on science has been largely concentrated on agriculture and related activities, which account for about two-thirds of public expenditure on research and development and three-quarters of the expenditure on scientific and technical information (STI) services. Thus, 35% of Ireland's total research and development, including the contribution by industry, is devoted to agriculture, reflecting the country's traditional dependence on farming. Research oriented toward increasing farm production has been predominant in the past, but food science and technology are now receiving special attention, with increasing emphasis on market research.

Expenditure on research and development in institutions of higher education has remained static for many years at 12–15% of the national total, percentages which are comparatively small by international standards. Only about 1.5% of total funds for research and development were devoted in 1971 to marine resources, this in spite of the extent and potential of the Irish coastline and continental shelf. Research and exploration in the marine environment is expensive and the involvement of EEC funds in this endeavour may be necessary if Ireland is to benefit fully from its situation in the Eastern Atlantic. The establishment, for example, of a European centre for marine science in Ireland would be a most welcome development. Ireland has also been late in discovering its mineral wealth and in realizing the need to invest heavily in scientific activities related to their exploration and exploitation.

Government expenditure on scientific activities, including research and development, is a matter for departments and the operational bodies within their sector. Thus, the vote of the Minister for Agriculture contains grants-in-aid for An Foras Talúntais, as well as a substantial sum for research within his own department; the Minister for Industry and Commerce provides in his vote for the Institute for Industrial Research and Standards while the Minister for Local Government's vote contains funds for An Foras Forbartha (The National Institute for Physical Planning and Construction Research). The Minister for Finance has sought the views of the NSC on the long-term programmes of some of the research institutes and this procedure has led to some reorientation and concentration of effort.

The bulk of the finance for university institutions is allocated by the Higher Education Authority (HEA), a newly established statutory body charged with coordination of expenditure on higher education. The authority is provided for in the vote of the Minister for Education. The National Science Council (NSC), provided for in the vote of the Minister for Finance, supports research projects of high scientific merit as attested to by referees, many of whom are abroad. Projects having economic and/or social potential, as well as scientific merit, are particularly encouraged. A bridging committee of the NSC and HEA has been formed to coordinate matters of common concern.

National Science Council

In December 1967 the government, following receipt of the joint Irish/OECD Report, *Science and Irish Economic Development*[8], established the National Science Council to advise it on matters of science policy. Members of the council are drawn from business, higher education and the public service; it reports to the Minister for Finance, who has responsibility for long-term economic development policy. The Minister stated at the inaugural meeting of the council that ". . . while all science is your concern, your attention as a council must be focused, at least for some time to come, on those aspects of science and technology which foster economic progress".

The council has undertaken the following activities, among others: publishing studies as a basis for science policy formulation and the selection of priority areas; interacting with and reviewing the medium and long-term programmes of the state-supported research institutes and other scientific bodies;

establishing a research grants scheme for universities and other institutions of higher education; formulating a scheme for research and development support to industry, which is administered by the Industrial Development Authority; communicating with scientific bodies in other countries and with appropriate international organizations with a view to bringing about greater Irish involvement in joint scientific projects.

As its four-year term of office drew to a close, the council after an evaluation of its own role, saw a need for change and made recommendations to the government concerning the powers and functions of a new body to succeed it[9]. The NSC was concerned that this body could ensure that technical programmes arrived at within the vertical departmental structure of government would be capable of dealing with the many horizontal problems, cutting across traditional departmental responsibilities, which face society today. It was also concerned that short-term financing of scientific programmes would be consistent with long-term policy for the attainment of national goals, while at the same time stimulating the creative drive of working scientists and of entrepreneurs in technology-based industry. The government accepted the advice that there should be established a statutory body with wider powers than those possessed by the present council, but postponed a decision on the detailed mechanism for implementing this decision. Meanwhile, the members of the first National Science Council were invited to continue in office.

OECD Review

Earlier the government, on the advice of the council, invited OECD to review Irish science policy, as well as policy for scientific and technical information. These two reviews were closely coupled as they proceeded in parallel; two separate "confrontation" meetings were held in Dublin in March of this year and full reports will be published by OECD in due course.

The country's overall economic and social policy formed a framework for the reviews. This calls particularly for the provision of industrial employment at an increased rate, as manpower employed in farm production continues to decline. In the long term, the examiners saw the much-needed increase in funds for research and development coming from the business enterprise sector, but in the meantime, and particularly as part of the strategy of preparing for full integration into EEC, they urged the government to consider increasing the level of its support for research and development, particularly that performed within manufacturing industry. They recommended that a state-sponsored advisory service be established to provide technological, management, financial and other relevant information for small and medium-sized firms.

The examiners see the higher education sector as having a crucial role in relation to the education of qualified manpower; it is here that they see the main potential for growth within the science system in Ireland. The achievement of this growth will, however, require energetic and skilful guidance from the resource allocating bodies, as well as the evolution of research policies within institutions of higher education.

The examiners found that Ireland, in common with many other countries, has neither a centralized science policy nor a coordinated policy for STI. They say that much of the difficulty arises from the limitation of the NSC role to a strictly advisory one, leaving it without effective powers of coordination.

The examiners agree that there is a need for a new statutory body to succeed the NSC and recommend the title National Board for Science and Technology (NBST). Its purpose would be to draw up and coordinate a central science policy. They commend recent approaches to detailed programming by the state-supported research institutes; in this way the information needed to make choices in future between different priorities and solutions in applied research and development will become available. The examiners emphasize the need to relate applied research and development closely to industrial and agricultural production and to relevant marketing intelligence.

Role of National Board

The NBST would formulate, under the direction of the Minister for Finance, a central science policy, and would prepare an overall science budget which would be presented to a Committee of the Cabinet for Science and Technology, but government departments would continue to have financial and other responsibilities for scientific institutes under their aegis. The NBST would have a special fund with which it would promote the filling of gaps in the national scientific effort. It would, through its influence on the science budget, including an STI sub-budget, achieve coordination and concentration of scientific activities, within the scientific community the establishment of focal centres or "centres of excellence" is widely acknowledged as desirable, but the implementation of such a policy is at present difficult, not least because of the uncoordinated funding of science and technology by government departments. The report envisages that a number of committees would report to the NBST; the STI examiners specify two such committees, one concerned with the coordination within the library network of documentation acquisition, storage and retrieval policies, the other with the coordination of technical information services, particularly those provided by government departments and the principal research institutes for users within individual sectors of the economy. Research into information techniques and the training of information specialists would be among the tasks of this latter committee.

The recommendations of the OECD examiners for the organization of science in the government sector go most of the way with the NSC proposals[10]. The chief difference is that, unlike NSC, the examiners would not give full powers for resource allocation to the new board, although they do concede that the need for such powers might arise at a future date—presumably if their proposed mechanism is not fully successful. They are concerned that relationships be strengthened between government departments having a need for a scientific input to policy making, on the one hand, and the operational scientific institutes on the other. Second, they see the need to maximize the impact of scientific activities on the output of the Irish economy. Their proposals are designed to attain those aims in the context of Ireland's involvement in EEC.

The legislation needed to establish the new statutory board for science and technology is currently being studied, and action by government and parliament is expected before the end of the year. Hopefully, the full potential of Irish science will then be realized for the benefit of scientists and of society alike.

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MANPOWER

International Mobility Among Scientists

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Non-governmental bodies have an important part to play in bringing about increased scientific mobility. International cooperation can gain very much from goodwill among scientists.

The intrinsically international character of science makes it logical that scientists from different countries should meet to share experiences, and compare methods in planning the further progress of science and technology. This is all the more important at the present time when science has a high responsibility to society, and when society must also act in a responsible way towards science.

Most international scientific cooperation is being directed to big projects, where the cost of equipment is high, and the duplication of facilities may be undesirable, and often impossible. Mobility among scientists—scientific interchange—is, however, perhaps the cheapest form of international cooperation, but it is also among the most effective. It is necessary because the scope and depth of scientific research today makes it impossible for most nations to provide all the facilities needed by their scientists. Of course, apart from any economic reasons, movement between countries has always been a recognized way of augmenting the training of research workers. The advantages do not rest solely with the individual, for on his return to his home country, his parent laboratory or institution gains from the broadening of his training and experience[1].

A few years ago, the Ministerial Conference of the Council of Europe adopted a resolution calling for more mobility in higher education and research in all fields[2]. It is needed, of course, in the human and social sciences as well as in the natural sciences, but most of all at present in the natural sciences and technology where the speed of development is greatest. This is a modern manifestation of the movement of scholars and teachers which occurred many centuries ago.

Taking a broad view, international mobility of scientists occurs in a number of ways, which include: attendance at research conferences; through participation in the affairs of the international scientific unions or similar bodies; through work at international research institutes of one kind or another; and through visits by individual research workers to foreign universities or research laboratories for short or long periods. Each of these mechanisms has its specific value, but in my view the exchanges of working research scientists may be the most effective in the long term.

Conferences

International scientific conferences can be large, involving a thousand or more participants; or small, with only a hundred or fewer people taking part. During the past twenty-five years, the number of large conferences has increased rapidly with a corresponding increase in cost. Certainly these meetings are often important and useful, but in my opinion their scientific value has become small, if judged against the cost.

By contrast, small specialized research conferences involving a hundred people or less, with a few selected main speakers who are expert in the field concerned, seem to me to have increasing scientific value; and especially if they take place in a relaxed atmosphere and when a previous decision not to publish the discussion stimulates free thinking and criticism. The Gordon Research Conferences of this type, started in the United States about twenty-five years ago, have proved outstandingly successful and have been followed in Europe during the past ten years by the EUCHEM conferences for chemistry, EUROMECH conferences in applied mechanics, and similar series in other fields, all organized voluntarily by international groups of working scientists themselves. There have been about fifty such small specialized EUCHEM conferences during the past eight years, covering a wide range of chemical topics, and held in different European countries. One is to take place in Spain this year. The intention and custom with such conferences is to operate at the minimum cost. Very often, they lead to continued contact and links between laboratories. The Council of Europe, and several private organizations, have given limited financial support to them, but they deserve to obtain greater and continuing help from public funds.

International Scientific Unions

During the past thirty years, many non-governmental international associations for different branches of science and technology have been established. They vary in purpose and effectiveness. Most of them are financed privately, but some receive help from governmental funds.

Perhaps the most significant is the International Council of Scientific Unions (ICSU) and its component unions, covering all the principal disciplines of natural science. Ninety-two countries now belong to the ICSU and its unions, being represented by their appropriate scientific body, usually the national academy, sometimes by a research council or other national organization.

It has been estimated that many (perhaps eight to ten) thousands of scientists from many countries are working voluntarily in commissions or groups of one sort or another for the ICSU and its unions. The unions themselves are concerned with work specific to their particular discipline. For example, IUPAC is concerned with international standards, nomenclature and terminology, agreement on analytical methods, criteria of purity of drugs and foodstuffs, and the compilation of reference data and chemical information. It has been advising the European Economic Communities for some years. It stimulates progress in chemistry by arranging symposia at intervals, and cooperates with other unions in a joint study of current problems in the teaching of science as a whole.

The ICSU itself conducts interdisciplinary studies on a world scale and it is also chief scientific adviser to UNESCO. In 1959, for example, it operated the global programme on the International Geophysical Year. During the past eight years, the International Biological Programme has examined the biological basis of human welfare. It has studied the facts affecting organic productivity, the potential use of natural resources, and human adaptability in changing conditions. The results are still being evaluated. A natural consequence to this was SCOPE, a special committee set up to study problems of man in his environment, some time before this matter became a topic for governments, leading to the Stockholm Conference of 1972. SCOPE has considered all aspects of pollution, identified some important factors for study and control, and explored the scientific problems of an ecological community. It continues to advise the agencies established at Stockholm, and is linked also with the new UNESCO programme, Man and the Biosphere.

These are just a few examples of ICSU's work. Of course, in spite of its global character, the movement of scientists involved is not excessive, for most of the work is done at national centres and coordinated at periodic meetings, or by correspondence. Yet there is a considerable exchange of information and thought internationally, which creates a sense of contact.

It is doubtful whether the important role of ICSU and its unions, not only for science but also in creating valuable personal relationships, is always appreciated sufficiently by governments. The administrative structure is small in relation to the work done, and it is important that as costs inevitably rise, the modest financial support which such organizations need shall not be lacking. It is also desirable that more younger scientists should be brought into this work. This is primarily a problem of money, although the amount needed would be, comparatively, very small.

International Facilities

A third way in which mobility of scientists may be involved is provided by the international research laboratories or institutes, which are usually established and supported by intergovernmental funds. Lately, there seems to have been a wave of enthusiasm for them in some quarters, backed by well organized pressure groups. Certainly CERN, the nuclear physics centre at Geneva, is acknowledged everywhere as a great success, and has enabled workers from many countries to be trained and carry out work which they could not otherwise have done. Marine biological stations, such as that at Naples, are also valuable because biological specimens required for the work are available at the location. The Trieste Institute for Theoretical Physics has also been successful, especially in providing a centre to which scientists from developing countries could come for a period. The recently established Institute for Insect Physiology at Nairobi also appears to be doing well.

In my opinion, however, great caution is needed before large international laboratories are established. They are necessary when the cost of equipment is too high for any single nation, and costs must be shared; or when particular facilities make the choice of a particular location essential. They may also be justified if there is no alternative mechanism for the training of scientists in the existing national centres. Yet, apart from the high expenditure required, they involve some dangers, and even a possible disruption of existing successful national research centres. Sometimes, too, the large national financial contribution which is required may have a serious impact on the total science budget in the country concerned, and lead to an unbalanced scientific development.

Interchange of Scientists

I now turn to my last example of scientific mobility, although I think it is almost certainly the most important. It is the interchange of working scientists between centres in different countries, for shorter or longer periods. In my youth, after taking a first degree at Oxford, I went to Berlin to do research work, and took a doctorate there. The enormous change and increased sophistication of modern research may now make movement at such an early stage of one's career much more difficult, and also less rewarding than it used to be. Exchanges at what may be called the postdoctoral level are more appropriate.

Exchanges can be either short term, say, up to two years, or longer term. Longer term mobility often involves a change of salaried employment in a place of higher education or research. In that case, a number of obstacles still remain, such as agreement about the equivalence of qualifications and eligibility for employment, the availability of work permits, the transferability of pension arrangements, sometimes in incompatibility of different national systems, the existence of restrictive national regulations, and difficulties of return later to the home country. Such difficulties need to be considered urgently by the international committees of university vice-chancellors and rectors, by national professional bodies, and by intergovernmental committees.

Short term exchanges are not subject to the same problems, and as I have said already, they are probably the cheapest among all major international scientific projects. Moreover, younger people can at once become involved in them. Visits abroad enable them to increase the rate of growth of knowledge, to enhance the quality and breadth of their training, to learn new techniques, improve their current awareness, and make contacts which strengthen the general scientific community. A leading French scientist remarked not long ago that the training of a research scientist is not complete unless he has spent a period, early in his career if possible, working abroad; and that he should later maintain contact with his professional colleagues in other countries. The fraction of the staff in universities and places of higher education and research who get this foreign experience is still not high enough.

Circumstances in Europe after 1945 were obviously not favourable for the movement of younger scientists. For this reason, there was a flight to North America, from which many did not return. Later, several agencies began to provide some support for exchanges, such as the national Research Councils in different countries, and intergovernmental bodies such as CERN, ESRO and more recently EMBO. Colleges and universities, however, even now have little money to promote such exchanges. Some private bodies, including the Humboldt Foundation and the Max Planck Society in Germany, provided travelling fellowships for scientists, although often only for foreigners to work in the country of the donor organization. Somewhat later, bodies such as the British Council and German Academic Exchange Service encouraged foreign exchanges at all levels, but the number of awards for research in the natural sciences and technology was insufficient.

A survey by the Royal Society of London in 1964–65 suggested that the total scientific exchanges in Europe was small, and not consistent with the resurgence of many excellent centres of research. After consultations with several of its sister academies and similar agencies in European countries, the Royal Society decided therefore to initiate a new scheme. This exchange programme started in January 1967, using private funds provided chiefly by the Ford and Wates Foundations. It provides for short study visits abroad, lasting a week to six months, and for fellowships lasting six months to two years. The response was so great that very soon the British government offered to make a more substantial grant to be used by the Royal Society, provided that a "balancing" mechanism could be established with the European partners. At the time, this "balancing" mechanism avoided difficulties of currency transfer, because the sending side paid the travel costs and the receiving side paid the subsistence, at agreed rates. This problem of currency transfer is less important now, but a partnership of this sort is still useful because the partners feel a joint responsibility and a continuing interest in the scheme.

The programme has grown rapidly, and today sixteen countries in western Europe are partners, namely: Austria, Belgium, Denmark, Finland, France, Germany, Greece, Ireland, Italy, the Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and Britain. The adhering bodies are the academies in Austria, Finland, Ireland, Italy, Norway, Portugal, Sweden and Britain; in Spain the Consejo Superior de Investigaciones Cientificas, in France the CNRS, in Germany the DFG, and corresponding organizations in the other countries.

This is a linked set of parallel bilateral arrangements in which each partner determines the scale of exchanges which it needs with the other partner, and can place emphasis on the particular fields of science that it prefers. Experienced scientists assess the applications, the sending side proposes and the receiving side is asked to approve, and the administration is minimal. There is little bureaucracy, quick decisions are made about applications for awards, and there is enough flexibility in the mechanism to ensure maximum success.

In six years, more than 1,500 awards have been made, covering all aspects of science and some aspects of technology. The number of awards has naturally been related roughly to the size of the country concerned. Not surprisingly, physics and chemistry are the most popular fields, but short visits for biological subjects seem to be very frequent. In 1972, the total sum spent was the equivalent of about £350,000. Last year, the British government decided to increase its grant to the Royal Society for this programme steadily during the next few years, so that if other partners are able to make corresponding increases, the sum available in 1975–76 will exceed £600,000.

This programme is operated essentially for scientists themselves, and the partners meet only on one day each year to discuss progress and plan for the following year. It was initiated through private funds, and although now supported by governmental grants, is non-governmental in character. A recent study has shown that the programme now holds a leading place among all scientific exchange schemes in Europe[3]. Its growth during six years has been impressive, and lends support to the suggestion made in a recent report that such exchange schemes are probably best carried out by non-governmental organizations[4]. The British Council has recently obtained increased funds for use in Europe and may be able to expand its own exchanges of this kind.

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FRANCE

Uncertainties in French Scientific Research

from *La Recherche*

With planning soon to start on the Seventh Plan, it is becoming clear that several of the policies enshrined in the Sixth Plan have been abandoned.

THE French elections of March 1973 have not had direct consequences for scientific research: Mr Jean Charbonnel remains at the head of the Ministère du Developpement Industriel et Scientifique, which has in its charge the Commissariat à l'Energie Atomique (CEA), the Centre National d'Etudes Spatiales (CNES), and the Delegation Générale à la Recherche Scientifique et Technique (DGRST); and Mr Joseph Fontanet continues to head the Ministère de l'Education Nationale, to which pertains the Centre Nationale de la Recherche Scientifique (CNRS). Questions of scientific research were almost totally absent from the electoral debates, and there is nothing to indicate, at least for the moment, that research policy is among the pressing preoccupations of the new Messmer government. The uncertainties about the future of French scientific research which were so abundantly reflected in the research budget for 1973, a "zero increase" budget, remain.

At present it is discussions concerning the orientation of the CNRS and the regulations governing its research personnel which are on the agenda. The role of the CNRS in basic research has been growing in recent years. With more than 6,500 researchers and a total budget of the order of 1,500 million francs, the CNRS has in fact increasingly taken on the role of a pilot organization for basic research. This situation poses some problems, because the resources devoted to research in the universities by the Direction des Enseignements Supérieurs of the Education Nationale have been proportionally curtailed for several years, and thus the CNRS now controls most of the resources for basic research. The management of CNRS wants, on the other hand, to improve its relations with industry, which would permit it to provide outlets for some of its researchers while addressing itself to problems of industrial research. This is a rather uncertain scheme, because it runs against the traditionally sceptical attitude of industrial people to university research. The CNRS has nevertheless increased its efforts in this direction during the past year, in laboratories working on the "engineer-

Mr Joseph Fontanet

Mr Jean Charbonnel

ing sciences", in order to develop certain lines of researches that have a close bearing on technological problems. This endeavour has been accompanied by the stated willingness of the directors of CNRS to redefine the regulations pertaining to its researchers. The principal objective sought in these reforms is to shorten the period of training for research (and therefore the time before submission of doctoral theses) and to make the end of this period into a turning point in the career of CNRS researchers, only the most capable being invited to pursue their careers in CNRS. Obviously, there is considerable opposition to such a reform, especially on the part of the researchers' unions, which see in these projects above all a means of institutionalizing penury. It is clear, however, that the debate about the education of researchers is quite fundamental, because the average age for completion of a thesis is now around thirty-one for researchers in the exact sciences. The future of basic research will depend to a great extent on the solutions which are found for these problems.

University research is also floundering in face of great organizational difficulties. The application of the 1968 law of Edgar Faure concerning the reorientation of the universities has in fact resulted, in those cases where both teaching and research is carried out, in a considerable excess of activity for teachers, and this seriously endangers the necessary equilibrium between teaching and research in a number of universities.

Atomic Energy

The other great problem of civilian research remains that of the broad scientific programmes in the realms of atomic energy, defence, and aerospace, which have long been considered as the chief motive powers of French science policy. The Sixth Plan has marked a very distinct evolutionary step in recommending a diminution of the share of these programmes in national defence research and development expenditure. The CEA has for its part started a move towards non-nuclear activities, with the setting up of an office for programmes of general interest and one for non-nuclear industrial cooperation. In 1972 the activities of these two offices represented 4% and 10% respectively of the civilian budget of CEA, and the directors intend to double the scale of such activities between now and 1980. It is clear that this attempt to realign part of the work of the CEA, which is pledged to put a ceiling on its budget (1,600 million francs in 1973 by comparison with 1,589 million francs in 1972), creates numerous problems, of which competition with other research organizations, public or private, is not the least.

Space Research

The situation at the CNES is in some ways similar to that of the CEA—especially as regards uncertainties about the future. French space policy, which had some successes during the 1960s, has not yet succeeded in finding a new orientation. In addition, French space policy is inevitably subject to the uncertainties of international space policy, because the international programmes of CNES account for about a third of its budget and thus exercise a serious constraint on its activities. It is well known that France has tried to persuade its European partners to construct jointly the rocket Europa III, but in the face of the reservations of its principal partners, Germany especially, France has prepared a modified project for the three-stage launcher L3S, which it may possibly carry out itself. (The cost of this launcher is estimated at 2,200 million francs against 3,100 million francs for Europa III.) No definite decision has been made about the L3S project (112 million francs have been designated in the 1973 budget for an eventual start), but it is clear that its financing cannot be borne solely by CNES. The departure from the Ministry of Defence of Mr Michel Debré, the most ardent advocate of the national launcher, raises serious questions about its future.

Meanwhile, CNES has to get by in 1973 with a total budget of 808 million francs, which is an increase of only 1% of that of 1972; this budget obviously does not permit it to engage in any new programmes.

Industrial Research

The Sixth Plan anticipated the according of a certain priority to industrial research, in particular to the procedures for stimulating such research by the state.

State support for industrial research, which represented 30% of the expenditure of industry on research and development in 1969, comes from several sources, in particular, the Ministère de la Defense Nationale, but it was specifically proposed to direct a greater proportion of state aid toward sectors such as mechanics, textiles, or chemical industry, which have up till now been neglected. The plan also recommended more prudence in the support to be given to the advanced technology sectors such as electronics. But in fact the recommendations of the plan have not been carried out by the government. Indeed, the money available for development aid has diminished by 50 million francs compared with 1972 and 85 million francs have been assigned to a "Plan electronique civil". This plan is designed to help a sector already "up", electronics, and is directed especially toward easing the difficulties of Thomson-CSF, contrary to the policy recommended by various experts. In continuing to devote an important part of its support for industrial research to the big companies the government is flying in the face of the policy of stimulating innovation in the small and medium-sized companies which was first recommended two years ago by the Ministère du Developpement Industriel et Scientifique. In this area of science policy also, there is evidently a gap between certain proclaimed intentions and the policy actually followed. Even though one may be anxious for the future of industrial research in certain areas, notably in those affected by mergers, it seems that the financing of industrial research by industry itself has grown regularly for several years, for it has come to represent 36% of the national expenditures on research and development in 1972 by comparison with 32.5% in 1969. Thus French industry has, it would seem, respected the Sixth Plan.

The preparation of the Seventh Plan is to begin within a few months, even though many of the objectives of the Sixth Plan have already been abandoned. It is clear that over and above the problems of the financing of research it is the problem of the functioning of the institutions of science policy which is already on the agenda.

THE NETHERLANDS

Dutch Science and TNO

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One way in which international cooperation in research and development can be fostered is through organizations like TNO. This article discusses the work of that body in the context of Dutch science generally.

WHEN thinking about international cooperation between countries in Europe the first things to note are the marked differences in culture, mentality, history and attitudes between the countries concerned. These differences are surprising, for the peoples of Europe have been mixing through the centuries. Yet, in comparing countries like Italy and the Netherlands one immediately sees that they not only differ in population and area but in particular also in mentality and in the entire attitude towards life. Even neighbouring countries like Belgium and the Netherlands have a very different way of life and of dealing with public matters. So, if I start this article by giving some numbers to compare the Netherlands with other Western countries it is with the proviso that these numbers reflect the special features of the country only partly and perhaps indirectly.

Holland has a relatively small population (13 million) but a high population density (400 km^{-2}). The level of science and technology may be described as high. This is not only the case in classic fields like civil engineering, especially building of bridges and waterworks, and agriculture, for the presence of Philips Industries means intense participation in modern fields of technology such as telecommunications, magnetic materials and computers. Given the small size of the country surprisingly many large industrial companies have their headquarters in Holland. As well as Philips there is also Shell, Unilever, AKZO, DSM and Hoogovens, to mention only a few.

The high level of technology is reflected in a large expenditure on research and development. As a proportion of GNP this was 1.9% in 1964, the same as France and surpassed by Britain (2.3%) and the United States (3.4%). If one considers only civil research and development, excluding military contributions, the Netherlands was nearly on a par with the United States (2.2%) in 1967 with 2.15% of the GNP and ahead of Britain (1.7%), France (1.6%), Germany (1.6%) and Japan (1.4%). Also as regards the total manpower in research and development as a proportion of the civilian labour force, the Netherlands (1.14%) was in 1967 second behind the United States (1.85%) and far ahead of France (0.99%) and Britain (0.91%) whereas technologically well developed countries like Sweden (0.67%) and Switzerland (0.78%) are even further behind. Of course, the small distances in the country are very favourable to the research and development climate which depends on intense communications between specialists of all kinds and ranks.

From its geographical situation and from its size the Netherlands has grown accustomed to think and act internationally in many ways. No industry can exist in Holland without a foreign market which is often many times larger than the domestic market.

Research Institutions

In a thorough report on the situation of research and development in the Netherlands[1] the OECD examiners expressed their surprise about the presence in the country of a number of large laboratories such as the Hydraulics Laboratory at Delft, the Laboratory for Shipbuilding in Wageningen, the Aerospace Laboratory at Amsterdam and the Reactor Centrum Nederland (RCN) in Petten, some of which have a good international reputation and attract many contracts from foreign countries. The examiners concluded that "it seems that these examples of national facilities reveal a difficult problem. How can a small industrialized country develop modern tools in research and development for sectors in which the critical mass exceeds the needs of the nation? The financial load may become prohibitive and, in addition, an unhealthy working climate may develop if no use is made of these facilities. International collaboration seems inevitable but with a plan for specialization that assures that each country is not developing the same tools. How can a public financial effort, conceived for a national purpose, be justified within such an international setting?"

Obvious as this statement may sound, something must be fundamentally wrong with it. Indeed the first three of the four laboratories mentioned above have developed their international reputation from selling their services throughout the world on the basis of bilateral contracts, whereas the RCN has from the beginning relied heavily on the formal international collaboration in the Euratom organization. The difficulties suggested by the OECD examiners have always been and still are much more evident in the case of RCN than of the other laboratories. This is, of course, a consequence partly of the unfortunate political troubles which gradually destroyed Euratom and partly of the particular course that the development of civil atomic energy has taken throughout the world. But in any case one should consider with caution and suspicion any all-too-easy conclusions from the OECD report such as "Before starting a high level research laboratory in any field, small nations should make sure this laboratory fits within a framework of formal international collaboration".

Generally, small nations are handicapped by their size in many ways, not only in research and development but also in industrial production, trade, services and even in environment control, because very often their home market is too small to support a full size enterprise and every foreign market can be closed or manipulated by government actions outside the control of the nation in question. The answer for the small nation is, however, not to wait until formal international collaboration has been established but to look for openings in the international markets and fill those vacancies with products of the highest attainable quality. This is, at least, the situation in the West today and very probably for the next few decades as well.

Like any handicap this one is also compensated by several factors. Once the necessity of international contacts has become apparent the whole world is open to the small nation as a potential market. With regard to research and development contracts in a small nation an institute is probably less hindered than it would be in a large country by objections from national industry to accepting contracts from foreign industry. The Dutch government-supported research organization TNO, which will be discussed more fully later in this article, accepts exclusive contracts from foreign industries and governments, after giving a right of first refusal to the national industry if there is an appropriate one.

Of course, the fact that in practice bilateral, contract-based international cooperation has so far been more efficient than formal international collaboration does not imply that this will not change in the future. Generally the Netherlands has played and will continue to play an active part in projects of international collaboration in research and development such as the ultracentrifuge project, the fast reactor project and others.

Once formal international collaboration has been started in a new field this may strongly affect the programme of national research institutions. It is therefore important that such institutions have sufficient flexibility to adapt themselves to changing circumstances, whether in the way of international collaboration or of a change of the market.

TNO Organization

The flexibility of a laboratory can be severely restricted by the "mission" it acquired at its foundation. If the market changes the original mission may become obsolete or less important whereas attempts to enter new fields may meet resistance from institutions with other missions. It must therefore be considered as a very fortunate decision of the Dutch government in 1932 not to found a research organization with a definite field of research but to found the Organization for Applied Scientific Research TNO (Toegepast Natuurwetenschappelijk Onderzoek) with the purpose "to foster the efficiency of applied scientific research", as it was stated in the TNO law of 1932.

At that time the importance of scientific research for the community at large was much less clear and accepted than today. Therefore the decision in 1932 must be considered as very far sighted. By the creation of TNO a meeting place was provided where scientists could look for information on the needs of the society and the community could look for advice from science. It is important that although the budget of TNO is guaranteed by the government the organization itself is independent, not forming a part of the government, and with employees of its own who are not government officials. In this way sufficient separation from political pressures and from involvement with government policy is maintained. Of course, government officials are members of the central board and most of the divisional boards of the organization, but they are always a minority among the representatives of industry, science and public institutions. So in the research and development policy of the government does not have very much effect on TNO although the ultimate financial control rests with the Ministry of Finance.

As stated earlier, one of the very strong points of TNO has always been that it is not mission oriented; any branch of science considered of sufficient importance in the context of application in any branch or activity of the community can be incorporated in the TNO organization without the necessity of founding new institutes, buildings and pension schemes. Although this situation has not remained completely unchallenged the existence of TNO it is still a strong point today in a world that is much more conscious of the importance of science for the community and changing much more rapidly than 40 years ago.

Part of the challenge to the freedom of TNO came from the necessity to organize research in units with a coherent purpose. This led first to the foundation of suborganizations of TNO (the NO for industry, trade and traffic, the GO for health, the VO for food, the RVO for defence and the LO, which later separated from the TNO organization, for agriculture. Under the auspices of each of these suborganizations there are several institutions, laboratories and working groups, each with a more or less specified mission. Yet the freedom of the organization as a whole to initiate or stop research in specified fields is much greater than would have been the case without the TNO framework.

Another challenge to the TNO philosophy has been the foundation of new mission oriented research institutions without taking advantage of the existence of TNO. Examples are the large National Organization for Agricultural Research which split off from TNO but still cooperates closely, and the Reactor Centrum Nederland, working in the field of nuclear energy, which also cooperates with TNO. The existence of institutions for applied research not belonging to TNO does not, however, strongly affect the freedom of TNO to choose its research projects. Of course, it means that the budget of TNO is only a part, and rather a small part, of the total effort in applied research of the country.

Table 1 Size of TNO

	CO (central)	NO (industry)	GO (health)	VO (food)	RVO (defence)	Total
No. of employees	711	1,853	749	430	654	4,397
No. of graduates	135	269	153	80	132	769

I shall now quote some figures. Table 1 shows the number of employees and of graduates in the organization. Table 2 gives the budget of the organization as a whole and of the suborganizations appropriately divided. Table 3 shows the expenditure on research and development in the Netherlands in 1971 divided according to source and destination. These figures are given in Dutch guilders, Hfl. 1 being about $0.34 (about £0.14). Table 3 demonstrates that the budget of TNO constitutes only a small fraction, in fact less than 1%, of the national expenditure on research and development and less than 35% of government expenditure on research outside the universities. Yet Hfl. 200 million is not a negligible sum and 4,400 employees including 769 graduates are not negligible either as a research team that can be directed quickly towards the solution of new problems turning up in the community.

To give an example, many TNO institutes from various suborganizations work on problems related to control of environment, while the central TNO organization has created a Commission for Environmental Research and a Study Centre for Environmental Research. These institutions are very useful in regulating the flow of information on environ-

Table 2 Budget of TNO, 1969 (Hfl. million)

	CO (central)	NO (industry)	GO (health)	VO (food)	RVO (defence)	Total
Direct government subsidies	11.7	40.7	17.5	8.0	25.9	103.8
Outside orders	7.9	19.9	4.5	4.2	1.9	38.4
Outside contributions	0.5	5.0	2.5	1.7	1.1	10.8
Total	20.1	65.6	24.5	13.9	28.9	153.0

Table 3 Expenditure on Research and Development in the Netherlands in 1971 (Hfl. millions) (from ref. 1)

I Government expenditure	
Education and science	629
Research and development missions, including TNO	488
International contributions	53
Industrial projects	70
Total government	1,240
II Industrial expenditure including government support of industry	
The "big five"	1,020
Others	700
Total industry	1,720
Total industrial expenditure excluding government support	1,650
Total expenditure on research and development	Hfl. 2.89×10^9
Total TNO budget	Hfl. 0.2×10^9

mental problems on the one hand, and on the results and prospects of research for the entire country on the other. Also in many cases where technical aid is requested by a developing country TNO can assist in finding or providing the technical specialists for the job. And in many international projects in which the Netherlands participates, TNO provides one or more national representatives.

I would by no means suggest that the TNO model for organizing research is advisable or the best for some or most other nations. But one can say that, in spite of much criticism which has been launched over the years, the Dutch are happy with the TNO organization as an efficient and flexible tool in adapting the research potential to the needs of the nation and are grateful to the remarkable foresight of those who founded it 40 years ago.

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[1] *Reviews of National Science Policies, Netherlands* (OECD, Paris, 1972).

GERMANY

Research in the Federal Republic of Germany

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The president of the German Research Society describes how scientific research is carried out and financed in the Federal Republic of Germany.

SCIENTIFIC research in the Federal Republic of Germany is carried out at government, semi-public and private research institutions. In addition, research is done to a limited extent by individuals, for example, for the annual "Research by Youth" competitions or in connexion with other projects encouraged by awards and prizes.

Government and Government-supported Establishments

In accordance with the Basic Law applying in the Federal Republic, national competences in the sector of science policy are divided between the Federal Government and the Länder. In accordance with Articles 30, 70 and 83 of the Basic Law, competence with regard to legislation and administration is in the first instance incumbent on the Länder. In accordance with Article 74, point 13, however, the Federal Government was, from the outset, equipped with extensive powers with regard to concurrent legislation, which were later, for example, extended to cover the field of the production and utilization of nuclear energy (Article 74, point 11a of the Basic Law). Parliamentary committees for dealing with science matters exist at parliamentary level both within the German Bundestag and also within the Länder parliaments.

At the Federal level there is a Cabinet Committee for Scientific Research, Education and Promotion of Training (Committee of Ministers), and at the level of government experts an interdepartmental Committee for Science and Research. Both committees deal with fundamental questions of science and research policy, and coordinate the Federal Government's promotion measures. The Länder governments have their Permanent Conference of Ministers of Education.

Administrative agreements have been concluded between the Federal Government and the Länder with a view to establishing a joint research policy. As a result of these agreements, joint bodies are appointed and financial matters are dealt with. By the Administrative Agreement of June 4 1964 (extended on February 8, 1968), a permanent commission was established by the Federal Government and the Länder for reciprocal information and the coordination of the financial promotion of science and research. An administrative committee was also created for the scrutiny and fixing of the grants to be provided annually by both the Federal Government and the Länder for the German Research Society and the Max Planck Society. The Science Council was also created as a result of an administrative agreement in 1957, as a joint advisory body of both the Federal and Länder governments, for dealing with scientific and research matters.

According to this administrative agreement, the Science Council has the task of drawing up an overall plan for the promotion of science. In so doing, it must also coordinate the plans submitted by the Federal and Länder governments with one another, and also establish priorities and degrees of urgency. Furthermore, the Science Council must present a programme of urgent tasks each year, and make recommendations as to how the promotion funds provided by both the Federal and Länder governments should be used. The Science Council is composed of members of the scientific and administrative commissions.

Since this year, responsibility at government level for science and research policy has rested with the Federal Minister for Education and Science, and also with the Federal Minister for Research and Technology, together with the relevant government departments. At Länder level, the ministers of education are responsible, in some cases the ministers for research, and the relevant Länder government departments.

All government competences from the pre-primary school to university, including vocational training, are in the hands of the Federal Ministry for Education and Science. This also includes the promotion of science at universities in close collaboration with the Federal Ministry for Research and Technology.

The competence of the Federal Ministry for Research and Technology embraces general research promotion measures—excluding the German Research Society and the centres of excellence which, on account of their link with universities, have been assigned to the Federal Ministry for Education and Science—research planning and coordination, technological research, development and innovation, nuclear research and technology, and competence for establishing principles and coordination for both civil and military aerospace research and development. In addition, the Ministry for Research and Technology has been given competence by the Federal Ministry of the Interior for the "data bank system" working group and also for the coordination of Federal Government information systems. The Federal Minister of Economics and Finance has transferred his competence for the promotion of electronic data processing to the Federal Ministry for Research and Technology, and finally the latter has received competence from the Federal Ministry for Youth, Family Affairs and Health for the "German Cancer Research Centre" foundation at Heidelberg.

In addition to both ministries dealing directly with education and research, promotion of research is dealt with in particular by the Ministry of Defence, the Ministry of Economics, the Ministry of the Interior and the Ministry of Food, Agriculture and Forestry. Environmental matters are, for example, concentrated at the Ministry of the Interior.

Federal Institutes

The Federal Government maintains—as do to a lesser extent the Länder—a number of its own research establishments. The organization of these establishments is a task for public administration, and each institute answers to the respective minister for the purposes of departmental research. Among these establishments are the Federal Health Office in Berlin, the Physico-technical Federal Institute at Brunswick, the Federal Institute for Materials Research in Berlin, the German Hydrographical Institute at Hamburg, the Agricultural Institute in Brunswick-Völkenrode, the Federal Institute for Regional Geography and Physical Planning in Bad Godesberg, the Federal Institutes for Hydraulics and Hydrography at Coblence, and the Federal Institute for Telecommunications Engineering at the Central Office of the Federal Postal Services at Darmstadt. These institutes do not restrict themselves solely to providing scientific aid for administrative activities and the tasks of the authorities, but to some extent also carry out fundamental research work. The work of several institutes has a direct bearing on industrial and technical development, as, for example, the Central Office for Telecommunications, or the Federal Institute for Soil Research, which devotes its activities to discovering and opening up deposits of raw materials in close cooperation with industry.

Autonomous Organizations Promoted by the State

Ten big science institutes have been created as autonomous organizations by the Federal Government and, in part, by the Länder. These institutes carry on scientific and technological research and development, to the extent that the execution of such work poses unusually great requirements as regards staff, funds and equipment exceeding certain limits. Among them are the Hahn Meitner Institute for Nuclear Research Ltd at Berlin (HMI), the German Electron Synchrotron at Hamburg (DESY), the Association for Heavy Ions Research Ltd at Darmstadt (GSI), the Nuclear Research Centre Ltd at Karlsruhe (GFK), the Jülich Nuclear Research Establishment (Ltd) (KFA), the Association for the Utilization of Nuclear Energy in Shipbuilding and Shipping at Hamburg (GKSS), the Max Planck Institute for Plasma Physics at Garching (IPP), the Association for Radiation and Environmental Research Ltd at Neuherberg (GSF), the German Aerospace Research and Testing Institute at Porz-Wahn (DFVLR), and the Association for Mathematics and Data Processing Ltd at Birlinghoven (GMD). The individual big science establishments deal with varying tasks. They are partly centres for basic research, partly research and development centres engaged on a large number of different projects, programmes and specialized areas.

In order to facilitate the desired possibilities of organization, the legal forms of the said government research establishments are, in addition to public institutes, non-independent institutes under public law, legal organizations under civil law, registered associations, companies with limited liability, or foundations having legal status. Where big science establishments are concerned, the form of limited liability company is chiefly chosen.

Academic Research

Academic research is carried out in a wide range of fields at universities by persons who work full-time or part-time there and make use of the equipment, means and services available. The universities are a matter for the individual Länder, and are either autonomous establishments of higher education, or in varying degrees of transition towards becoming part of larger organizations as comprehensive universities. Academic research serves to provide a scientific foundation for the selection of study subjects, the scientific further development of such subjects, the scientific dissemination of knowledge and as an introduction to research work (research-oriented learning). Non-application-oriented research (basic research), which can also be carried out cooperatively as project-oriented research or interdisciplinary research, is most suited to university purposes.

As a rule, academic research is carried out by research workers who receive emoluments from the government as civil servants or by government employees at government research establishments. Research at universities is distinguished from publicly supported extra-mural research in that legislation accords the universities extensive powers of self government. This is intended to guarantee considerable freedom of choice of subject and a diversity of orientations and methods for groups and individuals.

On the other hand, researchers at universities are usually engaged as teaching staff, meaning that they can only devote their energies to research in a limited degree. The compulsion to present methods and results provides both an excellent impulse for further development and ensures that scientific findings are made widely known. To the extent that university training has to be scientific, the institutional link with research is indispensable. In order to carry out research at universities, the following requirements must be met: the right to practise research as an integral part of the scientist's official duty to engage in scientific work; allowing the individual time in which to do research work; provision of the institutions with basic aids for research; and the right of the researchers to procure additional funds for research purposes.

Academic research varies. As a rule, however, it is organized as follows: a central level, with a Research Commission which is competent for distributing central research funds, and, in addition, is particularly responsible for the interdisciplinary orientation of the research institutes. Then there is the level of specialized branches of study, where the staff are primarily responsible for organizing the training offered. Within the disciplines, or parallel to them, are departments, institutes, working groups, project groups and similar bodies. The latter have their own budget and their internal self-administrative system, and, as well as carrying out training programmes, they are responsible for research.

In 1968, at the recommendation of the Science Council, a start was made on the establishment of centres of excellence at the universities in compliance with the respective applications from such universities. These centres of excellence are to provide more efficient research units at universities, facilitate intensified financial promotion and an efficiency survey, encourage cooperation between research workers and the various research institutions, and establish well planned priorities.

Max Planck Society

The Max Planck Society for the Advancement of the Sciences, which is financed chiefly by the Federal and Länder governments, carries out selective research in fields which are of particular scientific importance or "future oriented". The activities are pursued in the society's own special institutes, some of which are extremely large, and particularly high qualitative demands are made of the scientists working there. The natural science sector is given priority, and the bulk of the work done is devoted to basic research.

German Research Society

The German Research Society uses the funds made available to it by the Federal and Länder governments and the Donors' Association for German Science chiefly for the promotion of research projects drawn up by German scientists, and for the promotion of young scientists. In addition, the society supports cooperation among researchers and research planning in the Federal Republic. It counsels parliaments and governments on scientific questions, and in particular fosters the contacts between German research work and scientific activities in other countries too. Decisions are taken on a democratic basis by the experts elected by scientists and the representatives from the Federal and Länder governments. As a matter of principle, promotion is given to all sectors of science, and is directed first and foremost to research carried out at universities.

Fraunhofer Society for the Promotion of Applied Research

The Fraunhofer Society for the Promotion of Applied Research is a sponsoring organization in the sector of applied research. It has the task of facilitating the handling of scientific and technical problems of applied research, and, at the same time, of providing adequate scope for the initiative of individual scientists in this field (application-oriented basic research and contract research).

Academies

The five science academies in the Federal Republic (Düsseldorf, Göttingen, Heidelberg, Mainz and Munich) execute projects covering longer periods of time, exceeding the lifetime of a researcher. In addition, they constitute a forum for colloquia or symposia on topical scientific themes in which a small group of scientists participates.

International Participation

The Federal Government participates in a number of international organizations in the sector of research and development. A distinction must be made between organizations possessing their own research capacity (Euratom, CERN, ESRO, ELDO and so on), organizations with tasks in the field of promotion and coordination (for example, the OECD), individual international projects (for example, the Fast Breeder project and the Symphonie telecommunications satellite). International cooperation is in the throes of growth. Particular importance is attached to the fairest possible distribution of both benefits and burdens.

Non-governmental Research

This is industrial research, which must be defined as research aimed at creating new products. As a rule it is oriented to the goals of the individual enterprises, in order to guarantee their competitiveness and place in the market. It is devoted to research and development and aims at producing an economic profit. A distinction is made between the following. (1) Individual research carried out by enterprises themselves. This is the usual form of research pursued, and has been developed mostly by the steel, mechanical engineering and automobile industries, electrical engineering and the chemical industry. (2) The issuing of licences and specialization agreements, facilitating a kind of division of labour for the participating firms. (3) Industrial cooperative research carried out by research associations of branches of industry in jointly sponsored research centres, or as jointly financed contract research. Advisory committees composed of representatives of the member firms and of scientists discuss the research programmes which, in the opinion of both scientists and those engaged in industry, have priority for the respective branch of industry in each case. Development up to the production maturity stage is carried out by the enterprises themselves. Seventy such associations are grouped together in the Confederation of Industrial Research Associations (AIF) at Cologne. The industrial groups less oriented towards research (for example, mining, foodstuffs and semi-luxuries, leather goods, textiles, timber, paper and printing, quarrying, earths, glass) play the principal part in this. (4) Contract research is research which is carried out on the basis of an agreement concluded between the contract-awarding party and an individual scientist, research institute or another enterprise as the contractor. A special form is the procurement of expert opinions. Contract research can solve problems caused by bottlenecks in staff, funds or equipment. Contract research resulted in the establishment of the Battelle Institute and in the development of the Faunhofer Society as a sponsoring company. Cooperation between industrial research and government-supported research institutes can also be effected by way of consultancy contracts.

Funding of Research

In 1971, public expenditure on science amounted to DM 13,500 million. Of this sum, it is estimated that DM 8,000 million—1% of the gross national product—was spent on pure research projects. Research expenditure on the part of industry amounted to approximately DM 7,700 million, and was, therefore, almost as much as that spent by the government. Altogether, then, about 2% of the gross national product was spent on research and development.

Public funds, excluding private grants, contract fees and proceeds from capital, are distributed to a varying degree between the Federal and Länder governments. A rough estimate is shown in Table 1.

Table 1 Distribution of Funds for Research and Development

	Federal Government %	Länder or host land %
Federal research institutions	100	—
Länder research institutions	—	100
Big science establishments	90	10
Basic equipment for university research	10	90
Max Planck Society	50	50
German Research Society	50	50
Academies	—	100
International research institutions	100	—

Private expenditure on science stems chiefly from the industrial firms interested in such projects. In addition, donations and contributions in kind are given by both private individuals and private organizations and foundations for research purposes, for example, by the Donors' Association for German Science, by the Volkswagen, Fritz Thyssen, Bosch and Krupp Foundations, and organizations of a similar nature.

SPAIN

Spanish Science in a Dilemma

by our Special Correspondent

A meeting held in Madrid last month highlighted one of the chief problems with Spanish science—whether to concentrate limited resources on buying in technical knowledge or to establish a really viable Spanish research capability.

THERE is little doubt that Spain is making great efforts to improve its scientific image. Not the least of that country's attempts to improve the quality and organization of science was the recent meeting held in Madrid under the auspices of the association of scientists who work for the Consejo Superior de Investigaciones Científicas (CSIC), the Higher Council for Scientific Research. This meeting, which even a decade ago could not have been envisaged, focused attention on the problems of Spanish science by comparison with the way in which developed and other developing countries organize their science.

But what are the chief problems with the organization of science in Spain ? Clearly one of these is the shortage of money. Spain, in 1972, spent 0.4% of its gross national product on research and development, a figure similar to that spent by Iceland but which compares unfavourably with the 1.4% spent by Japan, 2.4% by Britain and a mammoth 3.0% by the United States, these latter figures being for 1970. In terms of hard cash Spanish industry in 1972 spent 5,000 million pesetas (£35.7 million) on research and development, 2,000 million pesetas more than it did in the previous year, which itself was double that spent in 1967. But all the same, Spain spent 13,000 million pesetas in 1972 in payments for foreign technology. Although this staggering deficit of 8,000 million pesetas did not increase between 1971 and 1972 it has gone up from a more modest value of about 6,000 million pesetas since 1967–68.

Where Research is Done

Traditionally, research in Spain has been carried out in research centres and not in universities, although the situation has changed to a certain extent in the past ten years. In 1967, according to the OECD[1], 52% of the total expenditure on research and development in the country was spent in government research centres, with the bulk of the remainder, 43.4%, being spent in industry. Only 3.2%—106 million pesetas in 1967—was spent in higher educational establishments, with 0.9% accounted for by research associations.

The shortage of money for research boils down to the basic fact that Spain spends as much a year on research within the country as it does in buying patents, licences and "know-how" from abroad. In this respect Spain compares most unfavourably with other countries. Figures for 1967 show that for each peseta spent on research and development in Spanish industry four pesetas were spent on licence payments. Italy, on the other hand, spent 30% more on internal research in industry in 1965 than it spent on external licences, while in 1967 Japan spent four times as much on industrial research as on licence payments. France, Germany, Belgium and Sweden all had a much healthier ratio of money spent on internal research to money spent on external licence payments.

The soul searching that is now going on within Spain is about whether or not Spain should continue to increase the sums it spends on external licences to improve rapidly the efficiency of its industry, basically following the plan which has made Japanese industry such a force to contend with in the past decade. It is accepted within the country that such a method of approach will be good in the short term, but what will be the long term effects ? Japan, in fact, spent much more on external licences in 1967 than any other country ($251.5 million, twice what it spent in 1965) but in both 1965 and 1967 it spent four times as much on industrial research at home.

On the other hand, it is recognized that provision of a substitute for knowledge imported from abroad would necessitate the spending of much more money on research. The equation is not, however, balanced by these factors for there is no doubt that an increase in home research will lead to better trained scientists and technologists and a better standard of research in the long term.

Which Way?

But time is fast running out and some decision must be made soon within Spain about which way the country must turn and how it should concentrate its efforts in the coming years. Last year a detailed plan for the reform of the administrative structure of research was called for, which is symptomatic of the realization within Spain that science and technology must play a large part in the future development of the country.

It is clear that Spain cannot afford to drop completely its dependence on foreign technology and there is therefore an evident need to establish priorities in some research fields. A balance must be established between creating technology within the country and importing knowledge from abroad, with the aim of eventually halting the increase in the sums of money spent on foreign technology. The question which must be answered is what fraction of Spain's limited resources should be spent on developing long term applied research at home and how much can sensibly be spent in buying technology to make Spanish industry immediately viable ?

Spain must also decide in the near future whether it is a good thing that most of the universities are divorced from meaningful research. Since the CSIC was set up in the late 1930s most of the pure research within the country has been carried out at research institutes attached to the Higher Council for Scientific Research. It is only in isolated instances that research groups have been set up in the universities, and this has happened only comparatively recently. But can a university fulfil its purpose without the involvement of the academic staff in research ? At the meeting in Madrid last month this question was aired several times. The purpose of the universities is to teach, said some participants who were employed at CSIC research institutes, and the research should be left to CSIC establishments. But, countered others, a university is a place for furthering knowledge and this cannot be done effectively without the active participation of the teachers in research. That the officials of the CSIC are aware of the problem became clear in Madrid when Dr Federico Mayor, the acting president of the CSIC, said that it was essential that the universities and the research council centres should be brought closer together.

University Outlook

There are at present twenty-seven universities in Spain, of which three are private establishments with the remainder being supported by the state. Three of these state universities, those at Santander, Cordoba and Extremadura were first funded in 1972 and 1973. In all about 250,000 students attend Spanish universities.

The teaching staff at the universities are, broadly speaking, divided into Catedraticos, Agregados and Adjuntos, which are the equivalent of professors, readers and lecturers respectively in British universities. There are about 1,500 Catedraticos and Agregados in the universities and more than 3,000 Adjuntos with something of the order of 2,500 to 5,000 staff lower on the scale.

Although most of the pure research in Spain is carried out at research institutes it is estimated that some 30 to 50% of the staff at the universities carry out research. But a great deal of this cannot be considered front line research. The funding of research within the universities comes from two sources. The universities themselves contribute about 500 to 1,000 million pesetas a year, while the Ministerio de Educacíon y Ciencia supports about 500 staff to the tune of about 250,000 pesetas each. Thus the university supports its own research to a much greater extent than the government does. The fellowship programme of the Ministry of Education and Science has been one of the successes of recent years in Spain. Each graduate student is now awarded 120,000 pesetas a year while the department in which he is studying is also awarded a similar amount by the government. In all about 900 million pesetas are spent supporting graduate students in this way.

But why has research in the universities been slow to build up in spite of encouragements offered to the staff in recent years? Perhaps the chief reason has been the relatively low salaries paid to university staff until comparatively recently. This forced a substantial proportion of the staff to spend only part of their time at the university. Another contributory reason was that the equipment available at the universities to carry out research was of a low quality and this did not offer any inducement to the staff to start research projects. But the position is now changing. The staff are better paid and money for research is more easily come by. Thus the university staff can be expected, as time progresses, to spend more and more time at the university. In particular the hope is that the staff in the medical faculties will spend more time at the university carrying out research—it is the members of these faculties which are mostly under attack for spending little time on the university campus.

Questions of Staffing

The traditional method of choosing staff for universities in Spain is by examination, but in the late 1960s an innovation was introduced that allowed universities to appoint some members of staff on a contract basis for periods of up to five years. In 1968 a new university, the Universidad Autónoma de Madrid, was set up and one of its features is that 90% of the staff are on contract; only 10% were appointed by the traditional method and thus have tenure. Other universities in the country also have staff on contract but they have many fewer such members than the Autonomous University.

But this innovation which was at first greeted with great enthusiasm in Spain and by Spanish scientists working abroad, is in danger of turning sour. For one thing the people on contract were first of all put on the same salary scale as tenured professors, readers and lecturers (Catedraticos, Agregados and Adjuntos), but since July 1972 the salaries of the traditionally appointed professors have been 30% higher than the salaries of those on contract. There is also concern, now that the initial five year period of the plan is coming to an end, that the contracts of some members of staff will not be renewed, or that they will be offered inferior terms.

There have already been difficulties at the Autonomous University in Madrid over the contracts of some junior people when they came up for renewal in October 1972. The new contracts presented were, according to the faculty members, unsatisfactory, but following discussions with the rector the contracts were changed. In March 1973, however, the staff members had still not received their completed contracts from the university authorities and they therefore took strike action last month. The strike was short lived but staff members who took part have been penalized by loss of salary in some instances and by suspension in others.

Needless to say, the effect on the morale of the staff on contract has been bad and it is sad to think that a successful experiment is in danger of failing because of administrative difficulties. There is little doubt that having staff on contract has enabled the Autonomous University to build up quickly viable research groups that are competitive with any in Spain—or any other country for that matter. It would be a tragedy for Spanish science if the research groups staffed by faculty members on contract at Spanish universities were made less effective because of the difficulties and problems of the past eight months.

The fear is that the scheme to appoint faculty members on contract will be abandoned and that Spanish universities will revert to choosing their faculties by the traditional methods. This would in all probability mean the splitting up of the larger departments built up under the new system into smaller and less effective units; one consequence would be that the import of new blood would be made more difficult. Ultimately those Spanish born scientists who have been seduced back to Spain by the advantages of the contract method of appointing university staff may once more leave their native land to practise their professions abroad.

Differentials

The administrative difficulties with Spanish science are, however, not all restricted to the universities, and a justifiable complaint of the scientists who work for the CSIC is that their salaries also are less than the salaries of the tenured staff at the universities. Another complaint, which emerged at the recent meeting at Madrid, is that some research institutes of the CSIC receive preferential treatment, and that while some institutes are struggling to carry out research on limited budgets others experience no such difficulties. But the reasonable answer to this, given by Dr D. Jose Llado, the deputy chairman of the CSIC, is that resources must be concentrated in areas where they will reap the greatest return—because of an overall shortage of funds—and this means that some institutes will be better off than others.

But the Spanish scientists must have a method of assessing whether or not the decisions taken to support one research institute to a greater extent than another are justified. More openness in decision making will be very much appreciated and the signs are that with the appointment of Dr Federico Mayor as acting president of the CSIC confidence will be built up in the administrators of science.

There is, however, little doubt that Spanish science could soon be a force to be reckoned with if the present trends are allowed to develop. The success of last month's meeting in Madrid is an encouraging sign. The discussion of science and science policy within the country that took place at the meeting shows that there are enough people prepared to debate the merits and disadvantages of various courses of action. This will ensure that the issues will at least have an airing before far reaching decisions are taken about the future of research and development within Spain.

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Oxidation of Acrylic Fibres for Carbon Fibre Formation

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The oxidation stage in the production of carbon fibres from acrylics is time-consuming. Here we describe why oxidation is necessary.

THE production of high strength and high modulus carbon fibres from any precursor requires the formation and retention of molecular orientation. Highly oriented acrylic fibres are very suitable starting materials because if certain precautions are taken during carbonization, chain scission and loss of molecular alignment occur to only a minor extent so that acceptable high strength and high modulus carbon fibres may be produced directly.

What are the precautions taken during the carbonization of acrylic fibres in order to prevent chain scission and loss of molecular alignment? The fibres are heated in an oxidizing atmosphere, while under mechanical restraint. It was the latter condition that enabled Watt, Phillips and Johnson to take out the patents for the production of high strength and high modulus fibres[1] after Shindo had previously produced carbon fibres from basically the same polymer system[2], without ensuring that molecular orientation was retained. But the question remained—why is oxidation so necessary?

Typical conditions for the preparation of high strength carbon fibres from 'Courtelle' acrylic fibres are: oxidation under mechanical restraint in air at 200° to 225° C for 2 to 10 h, followed by carbonization in vacuum or inert atmosphere, not under restraint, at a heating rate of 1° to 2° C min^{-1} to 1,500° C. These fibres may then be graphitized by heating at 1,800° to 2,500° C for 1 to 2 h under vacuum, which produces the high modulus fibre.

Here we describe an extension of previous work[3], an attempt to analyse by infrared spectroscopy and elemental analysis, the reactions occurring during oxidation.

Before the advent of high performance carbon fibres, acrylic polymers aroused considerable interest in the textile industry because of their thermal colouration reactions. Houtz, using the concept of ladder polymer structure, was the first to attempt an explanation of these reactions[4] (I) in which atactic polyacrylonitrile (I*a*) cyclized to give "ladder" structure (I*b*). After Houtz, most papers have referred to the formation of ladder polymer when acrylic polymers were "degraded" in inert, oxidizing or alkaline conditions[5–18]. There are, however, considerable differences in the proposed chemical structure of the ladder polymer, particularly after oxidation. Three principal structures for the oxidized polymer have been proposed.

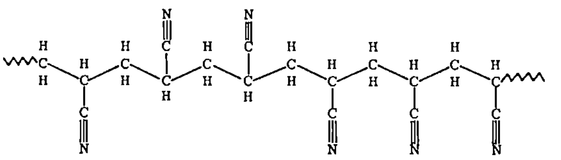
Structure II was suggested after infrared and elemental analysis of the oxidation, and shows oxygen present in the form of bridging ether type groups[15]. Structure III shows oxygen present only in the form of carbonyl groups. Evidence for this has been provided by model compounds, elemental analysis, infrared spectroscopy, mechanical properties and analysis of gases evolved during carbonization[27].

Structure IV shows each nitrogen atom donating its lone pair of electrons to an oxygen atom. This was proposed after a study of model compounds[9]. The conclusions of this report are not consistent with any of these formulae.

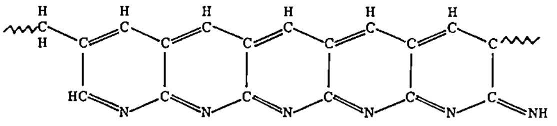
Two types of polymer fibres were used in this study—'Courtelle' and polyacrylonitrile (homopolymer). 'Courtelle' is stated to contain 6% methyl acrylate comonomer[19]. It may also contain about 1% of another vinyl monomer which acts as a dye site. Its stretch ratio is undeclared.

In contrast the polyacrylonitrile fibres were well characterized. Acrylonitrile was purified by distillation and polymerized using Redox free radical initiators to a conversion of 83%, and weight-average molecular weight of 220,000. The polymer contains acidic end groups and no attempt was made to fractionate. Fibres were spun from N-dimethylformamide solution using the wet spinning process with ethanediol coagulant. They were stretched eighteen times in two stages. Residual coagulant was evaporated off while the fibres were restrained on a frame.

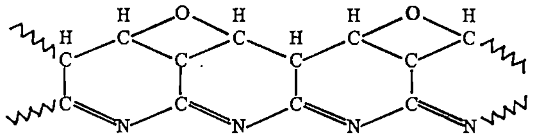
'Courtelle' fibres have an approximately circular cross-



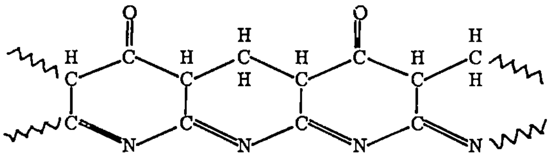
I*a*



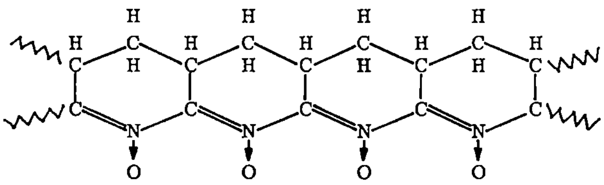
I*b*



II



III



IV

section of diameter 13 μm, whereas the PAN fibres had a kidney-shaped cross-section of mean thickness 10 μm.

Films of both polymers (thickness approximately 12 μm) were prepared by solvent casting and were used in the infrared analysis.

For oxidation the fibres were restrained on a rectangular tube frame (4 inch long) in an air circulating oven at 225° C. For treatment in nitrogen the frames and films were placed in a 200 ml tube under a flow rate of approximately 100 ml min^{-1} of nitrogen containing less than 0.1% oxygen.

A Perkin Elmer 420 Elemental Analyser was used to determine carbon, hydrogen and nitrogen contents: oxygen content was obtained by difference. Elemental analyses were carried out after various heat treatments and no account was taken of the escape of gaseous reaction products. It has been found that little weight change occurs during oxidation. Fiedler *et al.* showed that polyacrylonitrile fibres heated at 225° C for 5 h lose less than 2% overall weight[17]. This occurs while oxygen is being absorbed and water, hydrogen cyanide and ammonia are evolved. Watt has found that 3% of nitrogen is lost as hydrogen cyanide during the oxidation of copolymer fibres at 220° C (ref. 10). In the present work elemental contents are expressed in terms of the number of hydrogen, nitrogen or oxygen atoms per repeat unit, which contains three carbon atoms (Fig. 1).

The hydrogen content of 'Courtelle' precursor fibres is slightly greater than three atoms per monomer unit owing to the methyl acrylate comonomer. The loss of hydrogen is initially faster in 'Courtelle' than PAN, but after oxidation for 10 h the hydrogen content in both polymers is similar. The behaviour of nitrogen is nearly the same for both polymers and may be considered invariant. The oxygen content of 'Courtelle' is greater than zero owing to the methyl acrylate comonomer, and the slight oxygen content of PAN fibres may be a consequence of the acidic end groups. Measured by oxygen uptake, 'Courtelle' fibres initially oxidize faster than PAN fibres, but the oxygen contents after 10 h are very similar. The oxygen contents are better represented as the number of repeat units per oxygen atom (Fig. 2). When 'Courtelle' fibres are heated in nitrogen, there is no change in hydrogen, nitrogen or oxygen contents in the repeat unit (Fig. 1). These results indicate that, apart from differences in rates of reaction, there is little qualitative difference in the reactions between the two polymers.

After 5 h oxidation at 225° C, the repeat unit in 'Courtelle' should contain on average one hydrogen atom less, the same number of nitrogen atoms and one oxygen atom per two to three repeat units. After heat treatment for 5 h in nitrogen, the repeat unit should contain the same ratio of the elements as the precursor.

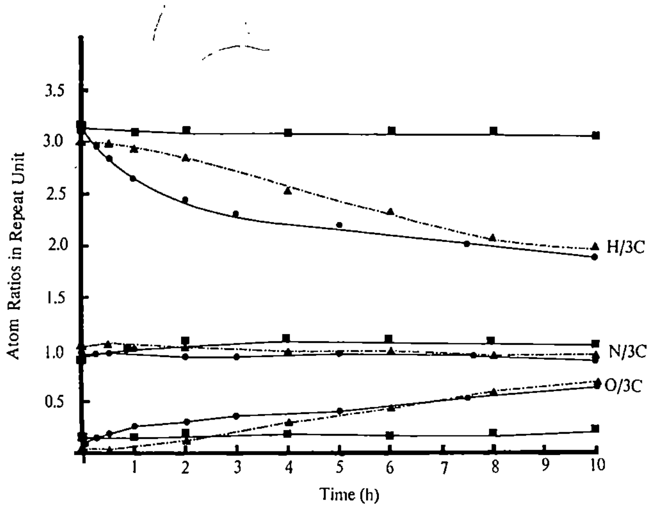


Fig. 1 Atomic ratios in repeat unit during thermal treatment (225° C) in air and nitrogen. ▲— · —▲, Polyacrylonitrile fibres —air; ●—●, 'Courtelle' fibres—air; ■—■, 'Courtelle' fibres —nitrogen.

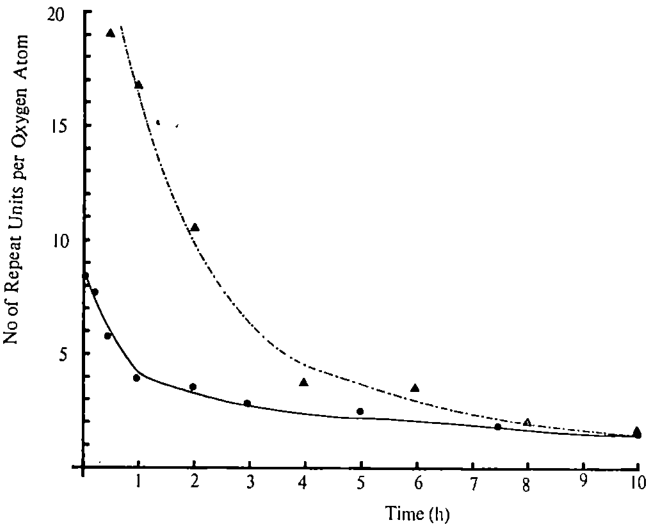


Fig. 2 Number of repeat units per oxygen atom during oxidation (225° C/air). ▲— · —▲, Polyacrylonitrile fibres; ●—●, 'Courtelle' fibres.

Qualitative Infrared Analysis

In the spectrum of polyacrylonitrile (Fig. 5*a*), the absorption peaks of particular interest are due to CH_2 (2,940 cm^{-1}) and $C\equiv N$ (2,240 cm^{-1}). The spectrum of 'Courtelle' (Fig. 3*a*) contains peaks additional to those in PAN; $C=O$ (1,730 cm^{-1}) and $C-O$ (1,170 cm^{-1}).

The spectra obtained from the fibres directly, using the KBr disk method were more satisfactory than those using the attenuated total reflectance method, although the spectrum between 4,000 cm^{-1} and 3,000 cm^{-1} was obscured because of a particle radiation scattering effect and residual moisture in the KBr.

When 'Courtelle' fibres are oxidized the reduction in intensities of peaks at 2,940 cm^{-1} and 2,240 cm^{-1} indicate that the CH_2 and $C\equiv N$ groups are reacting (Fig. 3). Also, new absorption peaks are created at 1,590 and 800 cm^{-1} and a shoulder forms at 2,180 cm^{-1}. After several hours of oxidation a broad absorption is created between 1,800 cm^{-1} and 1,000 cm^{-1}. With 'Courtelle' film (Fig. 4*a*) some changes also occur in the 3,400–3,000 cm^{-1} region. Otherwise the spectra taken of film and fibres are very similar.

The absorption at 2,180 cm^{-1} is probably caused by a nitrile group which is in electron resonance with another part of the molecule. In such circumstances it is a well known phenomenon that the frequency of vibration is decreased.

The absorption at 1,590 cm^{-1} may be assigned to $C=C$ and/or $C=N$ groups, and as it would have an important bearing on the formation of carbon fibres, this point is worth further discussion. If $C=C$ bonds are formed in this heat treated PAN system, only two types of substitution on the $C=C$ group are likely, $R_1R_2C=CR_3R_4$ and $R_1R_2C=CHR_3$, both of which would be expected to absorb between 1,675 cm^{-1} and 1,665 cm^{-1} (ref. 20). Conjugation and aromaticity, however, would be expected to shift the frequency of a particular absorption to perhaps 1,450 cm^{-1}. Another possibility is the formation of an $R_1R_2C=C=N-R_3$ group, but this is considered very unlikely. Similar $X=Y=Z$ systems have been found to absorb between 2,130 cm^{-1} and 1,900 cm^{-1} (ref. 25) where we found no absorption.

If the $C=C$ group carries a hydrogen atom, the $=C-H$ group would be expected to absorb in three regions because of the stretch, in-plane bend and out-of-plane bend vibrations. The stretch vibration would be expected to occur with medium intensity at 3,030 to 3,000 cm^{-1} whether the double bond was alkene or aromatic in nature. The in-plane bend vibration

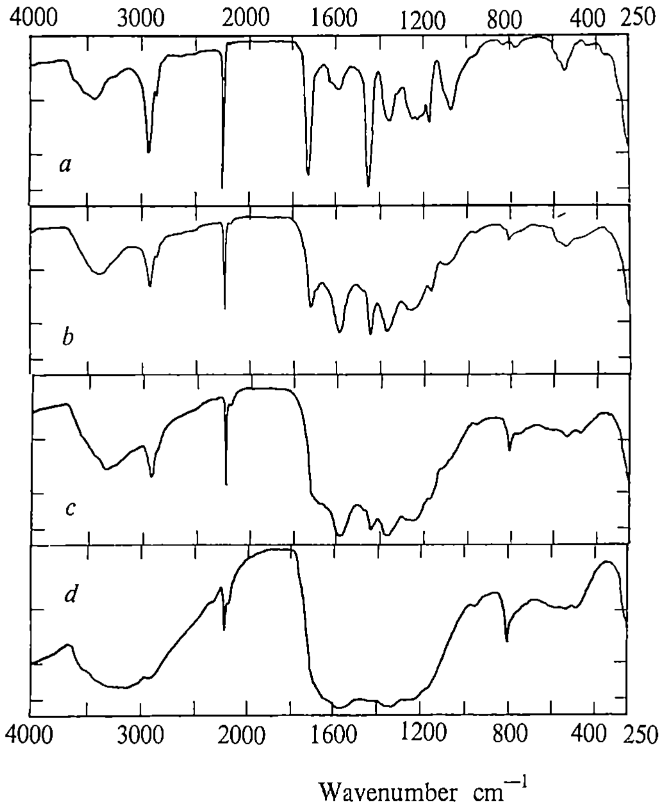


Fig. 3 'Courtelle' fibres heated in air at 225° C for *a*, zero; *b*, 0.25 h; *c*, 1.25 h; *d*, 5 h.

usually occurs at 1,450 to 1,250 cm^{-1} in alkenes and 1,250 to 1,000 cm^{-1} in aromatic molecules. The use of these absorptions in qualitative analysis of polymer systems is generally unreliable owing to their weak intensity and confusion through overlap with C–C and C–O stretching vibrations. Additionally, these vibrations can be confused with ring vibrations in 6-membered aromatic molecules, for instance pyridine derivatives absorb strongly at 1,250 to 1,000 cm^{-1} whereas the corresponding quininoid molecules show no strong absorptions in this region[23]. In this polymer system an alkene out-of-plane =C–H vibration would be expected to absorb strongly at 840 to 790 cm^{-1} whereas the corresponding vibration in an aromatic molecule may be found within a larger range 900 to 700 cm^{-1}.

In practice an intense absorption occurs at 1,590 cm^{-1} while a shoulder develops at 1,655 cm^{-1}. No significant absorption occurs at 3,030 to 3,000 cm^{-1} while an intense peak develops at 800 cm^{-1}. There seem to be no characteristic features on the absorption 1,550 to 1,000 cm^{-1}. So although there is evidence for the formation of carbon carbon unsaturation, a more intense absorption in the region 3,030 to 3,000 cm^{-1} at least, would have been expected.

At this stage additional information is provided by comparing the spectra of 'Courtelle' films which have been heated for similar periods in air and nitrogen (Fig. 4). It can be seen that whereas in the oxidized film peaks are produced at 3,330 cm^{-1} and 3,200 cm^{-1}, in the spectrum of the nitrogen treated film the peak at 3,330 cm^{-1} is reduced to a shoulder. This shoulder is further reduced in the corresponding spectrum of polyacrylonitrile film (not shown). Thus it seems that oxidation causes the formation of OH and NH groups whereas heat treatment in nitrogen produces NH groups and only small quantities of OH groups which probably originate from the oxygen in the copolymer molecules in 'Courtelle' and the end groups in polyacrylonitrile. The hydrogen required for the formation of NH groups when there is no oxidation must come from degradation products, indicating that NH groups are formed at the ends of cyclized sequences.

Another important difference is the virtual absence in the spectrum of the nitrogen treated film of the peak at 800 cm^{-1}. This result is also reported elsewhere[24]. Again this peak is reduced in the corresponding polyacrylonitrile film and is probably caused by oxygen in the polymer molecule. Thus it would seem that C=C groups are not formed to a significant extent when oxygen is absent and further evidence is provided by the retention of the CH_2 absorption at 2,940 cm^{-1}. But, equally important is that the 1,590 cm^{-1} is still present. This fact, together with the reduction in intensity of the nitrile peak (2,240 cm^{-1}) shows that C=N groups are being formed in the film heated in nitrogen. The nitrile peak, however, also decreases in the oxidized film so that it can be assumed that in this case also C=N groups are being formed. Further evidence is provided by the strong =C–H peak at 800 cm^{-1} which in the oxidized film develops much slower than the 1,590 cm^{-1} peak.

The formation of C=N groups is indicative of the development of heterocyclic structures. Other evidence is provided by thermal analysis[8] and the observation, first described by Houtz[4] that after heat treatment the polymer has greatly improved thermal stability.

The oxidation of polymers often results in the formation of carbonyl groups, but the spectrum of 'Courtelle' contains a strong carbonyl absorption from the comonomer. It can be seen from the spectra of PAN film during oxidation (Fig. 5) that carbonyl groups (1,710 cm^{-1}) are formed on oxidation. Apart from carbon carbon unsaturation, the shoulder at 1,650 cm^{-1} could be attributed to a carbonyl group in a β-diketene. Both these absorbances are absent in the spectrum of the polymer that was heat treated in nitrogen.

Previously, an empirical formula for the oxidized polymer was developed from the results of elemental and infrared analysis, and the assumption that oxygen was present only in the form of bridging ether groups[15] (structure II). Although the present work does not allow us to comment on the possible formation of bridging ether groups, it has shown clearly that oxygen is present in the oxidized polymer in hydroxyl and carbonyl groups, and there is considerable evidence for the formation of fully aromatic heterocyclic structures; heat treatment in nitrogen leaves the carbon backbone chain relatively unaltered, however, whereas the heterocyclic structures are still formed with conjugation between the C=N groups.

It is desirable to take quantitative measurements for the fibres from their spectra. This is not possible owing to the uncertainty of equating, between the KBr disks, the amount of material which is absorbing radiation. It is, however,

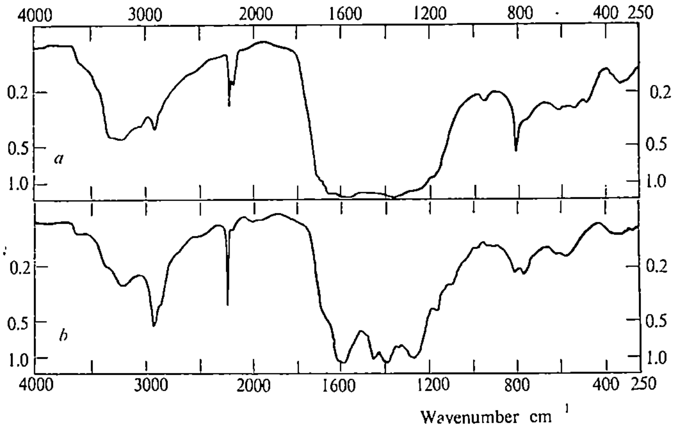


Fig. 4 'Courtelle' film heated at 225° C for 9 h in *a*, air; *b*, nitrogen.

possible using film provided the assumption is made that the thickness of the film does not change during the treatment.

In order to use the measurements made on the film to give information about the fibres, a parameter has to be used which is independent of the absorption characteristics of the particular form of the specimen. For both fibres and film, graphs are drawn of the change of this parameter with heating time. . Then, for any particular value of the parameter, the fibres and film are said to be equivalent at their corresponding heating periods as found from the graphs.

The parameter which has been used for 'Courtelle' fibres and film is the ratio of intensities of absorptions at 2,180 cm^{-1} and 2,240 cm^{-1} (Fig. 6). These have been chosen because their baselines are most easily determined. 'Courtelle' fibres which have been oxidized for 5 h at 225° C in air are found to be equivalent to the film which had had 8 h oxidation. In this film, and therefore in the fibres oxidized for 5 h, five out of seven of the original nitrile groups had "reacted". In this sense "reacted" means either to have become conjugated or have actually reacted to a C=N group.

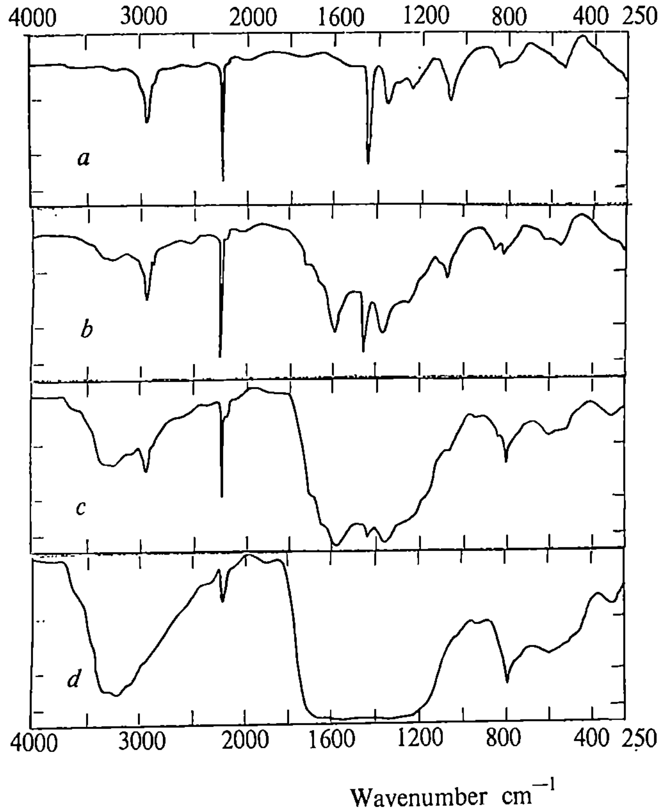


Fig. 5 Polyacrylonitrile film heated in air at 225° C for *a*, zero; *b*, 4 h; *c*, 10 h.

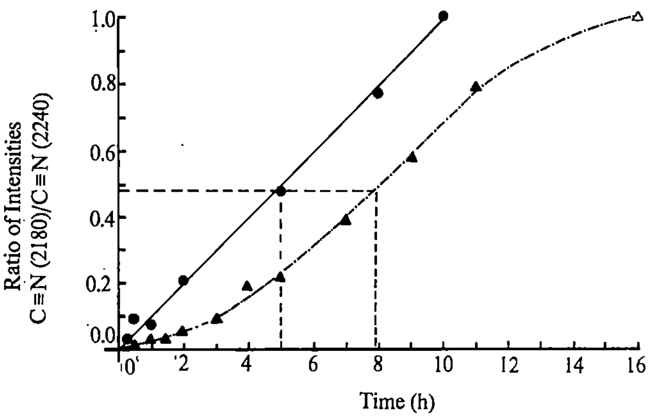


Fig. 6 Correlation between 'Courtelle' fibres and film during oxidation at 225° C in air. ●, Fibres; ▲, films.

Using the same method for 'Courtelle' fibres and film which had been heated in nitrogen, it was found that in fibres which had been heated for 5 h, one out of two of the original nitrile groups has "reacted".

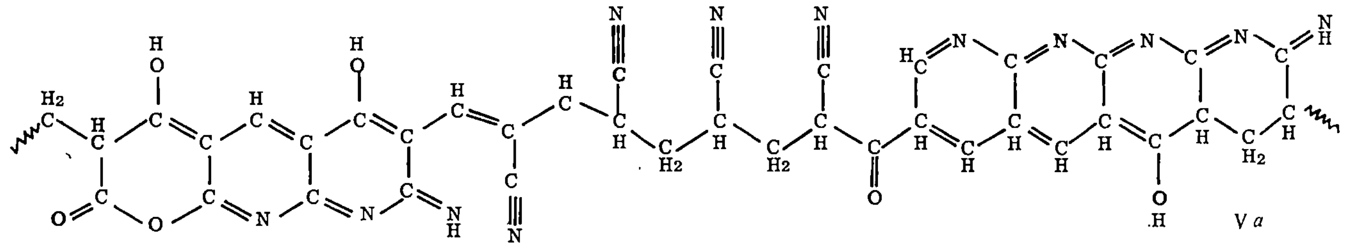
Unfortunately, no method has been found for accurately measuring the length of the cyclized sequences, using infrared spectroscopy.

Chemical Formulae for the Heat-treated Polymer

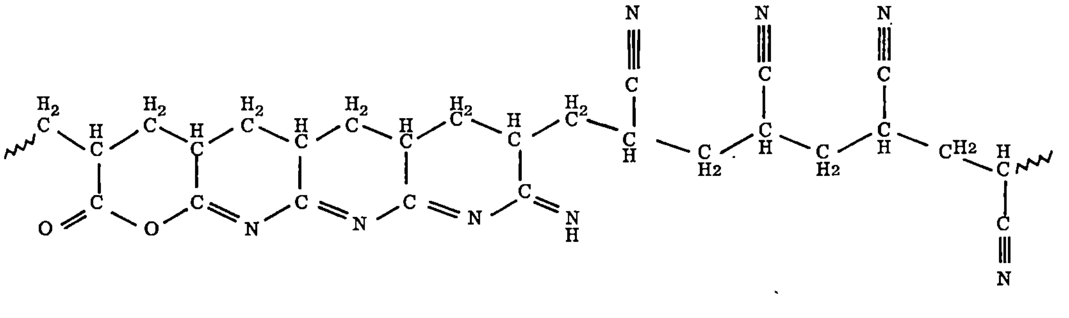
We are interested, not in the formula of the fully oxidized polymer, but of 'Courtelle' fibres which have been heated at 225° C in air for 5 h. In these conditions both elemental analysis and infrared spectroscopy indicate that only partial oxidation has been achieved.

The following quantitative parameters have been determined: the ratios hydrogen, nitrogen and oxygen to carbon, and the ratio of the number of the original type of nitrile groups remaining to the number that have "reacted". These parameters have been incorporated in the proposed chemical formulae of both oxidized V*a* and non-oxidized fibres V*b*. Otherwise these formulae are merely a possible collection of the chemical species shown to be present, and no inference may be made regarding the order of the groups. Also hydrogen bonding possibilities have been omitted for clarity, but it is likely that both inter and intramolecular hydrogen bonding is present.

It has been shown that comonomers containing carboxylic



V *a*



V *b*

groups can initiate the cyclization reactions[13], and this feature is included in the formulae.

Implications

When 'Courtelle' fibres are heated in air, two basic types of reaction occur, oxidation and cyclization. Cyclization also occurs in fibres which have been heated in an inert atmosphere.

By comparison with the cyclized sequences in the nitrogen treated polymer, those in the oxidized polymer are stiffer and more thermally stable because they are aromatic and have greater bond energies. The oxygen in the oxidized polymer is present as hydroxyl groups, which are substituted into the main chain probably in β positions, and carbonyl groups.

The morphology of the oxidized molecule can be considered to be stiff, planar cyclized rings connected by relatively mobile linear segments. Different cyclized sections will be in different planes. When the molecule is heated in the early stages of carbonization, the linear segments will twist allowing different cyclized sequences to become planer, so laying the foundations of the graphite structure. The driving force for this alignment could be a condensation reaction between the hydroxyl groups: water has been analysed in the evolved gases at early stages of carbonization[6,17].

It would seem that oxidation is necessary for at least two reasons. It allows the formation of conjugated and aromatic structures which are more capable of withstanding the temperatures of carbonization without molecular breakdown. Crosslinking through condensation reactions in the early stages of carbonization further stabilizes the structure. If oxidation is omitted the cyclized segments are not aromatic: only the $C{=}N$ groups are conjugated. Thus the bond energies in the ring will be lower, allowing more possibility of chain scission. The absence of hydroxyl groups may result in the non-alignment of different cyclized sequences. Both effects will allow a relatively disordered structure to develop, and the formation and retention of molecular orientation, referred to at the beginning, will have been lost.

We can consider the oxidized fibre as the precursor fibre, and use it as a model for other precursor fibres. We can say that in general the precursor fibre must be thermally stable. This means we must build into the polymer molecule electron delocalization and aromaticity. But these are the very properties which must be excluded in order to be able to stretch the fibre and produce a high degree of molecular orientation. We suspect therefore that it will not be possible directly and easily to produce a carbon fibre with good mechanical properties from thermally stable polymers. We must resort to producing a linear plastic polymer with poor thermal stability, which can easily be softened and stretched to produce the required molecular alignment. Then we must carry out a chemical reaction to convert the polymer molecules into cyclic aromatic structures without loss of molecular orientation.

We thank Dr B. C. Stace for his advice and assistance.

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Double Helix at Atomic Resolution

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The sodium salt of the dinucleoside phosphate adenosyl-3′,5′-uridine phosphate crystallizes in the form of a right handed antiparallel double helix with Watson-Crick hydrogen bonding between uracil and adenine. A sodium ion is located in the minor groove of the helix complexed to both uracil rings.

MANY important functions in molecular biology are determined by antiparallel double helical nucleic acids with complementary base pairing between the two strands, as first described by Watson and Crick[1]. Although considerable work has been carried out on the structures of double helical nucleic acids[2–8], the fine details of their molecular architecture have never been available at atomic resolution. The X-ray diffraction patterns shown by fibres of DNA and RNA characteristically die out at resolution greater than about 3 Å. Because of this, there has been considerable discussion[9–13] about the actual nature of the detailed stereochemistry and hydrogen bonding in double helical DNA. One way to obtain additional information about these atomic details is to crystallize oligonucleotide fragments of known sequence which may form double helices in the crystal lattice. Here we report the single crystal X-ray analysis of the sodium salt of adenosyl-3′,5′-uridine phosphate (ApU) which forms a right handed antiparallel double helix in which the ribose phosphate backbones are held together by Watson–Crick hydrogen bonding between the adenine and uracil residues. This is the first crystal structure in which the atomic details of double helical nucleic acids can be visualized. In addition, this is the first single crystal structure showing Watson–Crick base pairing between adenine and uracil.

Experiments

The sodium salt of ApU (Miles) was mixed stoichiometrically with bromotertiary butyl amine in a 40% solution of 2-methyl-2,4-pentanediol (MPD). A small volume of the solution was placed in equilibrium with a large reservoir of 60% MPD in a closed container which was stored at 4° C. After standing for 2 weeks, small prismatic crystals with well defined faces began to appear. Crystal growth continued slowly for several months and yielded crystals suitable for X-ray analysis. The amine was put into the solution in the hope that it might become the cation of the structure; however, subsequent analysis revealed that the sodium salt had crystallized rather than the bromoamine. A crystal measuring 0.2 mm×0.15 mm×0.05 mm was mounted on the tip of a glass fibre for X-ray analysis. The crystal was found to be monoclinic, space group $P2_1$, with cell dimensions $a=18.025$ Å, $b=17.501$ Å, $c=9.677$ Å, $\beta=99.45°$. The crystal density measured in a density gradient was 1.53. In order to obtain a calculated density near the observed density, it was necessary to assume four molecules of Na^+ApU^- and twenty-two water molecules in the unit cell. This surprisingly high degree of hydration was subsequently shown to be a low estimate as solution of the structure revealed twenty-four water molecules in the unit cell. Three-dimensional X-ray diffraction intensity data were collected out to a resolution of 0.8 Å, with a 'Picker' FACS-1 diffractometer in an 'Omega' step scan mode, using Nickel filtered CuKα radiation. The data were collected at 8° C and were corrected for Lorentz and polarization effects; no absorption correction has been applied because of the low mass absorption coefficient (18.0).

Solution of the Structure

Although there was no crystallographic two-fold axis in the lattice, the presence of a peak nearly 40% the height of the origin on the Harker section of the Patterson function suggested a non-crystallographic two-fold axis in the structure. From this we inferred the double helical nature of ApU. This peak was assumed to contain all vectors from each atom of one ApU molecule to the corresponding atom of the other independent molecule, symmetry related. From previous work with the protonated dinucleoside phosphate uridyl-3′,5′-adenosine phosphate[14] (UpA), it was known that resolution difference Patterson techniques were effective in discriminating vectors arising from two second row atoms in a large structure. (Resolution difference Patterson techniques are those in which two Patterson or superposition functions, for example, multiple minimum functions or N-atom symmetry minimum functions are compared. These two Pattersons are (1) the standard F^2 Patterson, calculated using all the diffraction data, and (2) a Patterson calculated only from the higher order reflexions whose Fs should contain a larger contribution from the heavier atoms sought as they are relatively denser near the atomic centres. The origins of both Pattersons are normalized to the same value. Peaks arising from heavy atom-heavy atom vectors ought to be relatively more prominent than overlapping light atom-light atom vectors in the second map. In this work, the first Patterson contained all the 1 Å data, and the second function contained the data in the shell between 1.5 Å and 1 Å.)

UpA contained seventy-seven first row atoms. Thus, we hoped to locate the phosphorus atoms by using this vector as the basis vector of a Resolution Difference 2-Atom Symmetry Minimum Function[15,16] (RDSMF(2)). The RDSMF(2) initially indicated the wrong location for the phosphorus atoms, however, which was obvious when Fourier refinement procedures failed to reveal the structure. An (E^2-1) Patterson (E is the quasinormalized structure factor) was therefore calculated and the map and its Fourier coefficients were corrected for non-negativity and minimal bond lengths according to the procedure proposed by Karle and Hauptman[17]: (1) all negative points in the Patterson map and all points within a 0.9 Å radius of the origin were zeroed; (2) the map was Fourier transformed and all resultant (E^2-1) coefficients less than -1 were raised to -1; (3) a new Patterson map was calculated from these coefficients, and the process was iterated until convergence was obtained at forty cycles. From the final amplitudes, a set of thermally sharpened Fs were generated, and used to calculate Patterson functions. The RDSMF(2) calculated from these Pattersons revealed the proper phosphorus locations. Approximate phosphate orientations were derived from Patterson superpositions. Fourteen cycles of Fourier refinement using the corrected Es as Fourier amplitudes revealed the two molecules of ApU, the sodium ions and four water molecules. The other water molecules were located in a series of difference syntheses. The structure has been refined using isotropic thermal parameters and only the 1 Å observed data by full matrix least squares. The current R factor is 0.091. (The discrepancy factor, R, is defined as $R=\Sigma||F_o|-|F_c||/\Sigma|F_o|$, where $|F_o|$ and $|F_c|$ are the amplitudes of the observed and calculated structure factors, respectively.) Because this is one of the larger non-centrosymmetric biological crystal structures solved without isomorphous replacement, we will expand on the details of the solution in a later publication.

Structure

In the analysis of this structure, there are two striking features. The non-crystallographic pseudo two-fold axis mentioned above rotates one ApU molecule into the other, so that the structure forms a segment of a right handed antiparallel double helix in which the bases are hydrogen bonded to each other in the Watson–Crick manner, as shown in Figs. 1 and 2. This is the familiar hydrogen bonding between adenine and uracil which is believed to occur quite generally in double helical nucleic acids. Nonetheless, this type of hydrogen bonding had never been seen previously in single crystal X-ray analysis. It should be noted that the two-fold axis to which we are referring lies half-way between the base planes, rather than in those planes. Axes in the planes are the ones usually discussed in the literature, but the periodic nature of double helices generates symmetry elements at both locations.

The other prominent feature was that the crystal was heavily hydrated, so that the ApU molecules are surrounded by large numbers of water molecules. This observation, coupled with the non-crystallographic nature of the two-fold axis, leads us to believe that the double helical nature of ApU is a function of the molecules themselves, rather than crystal packing forces.

The separation between the base pairs is 3.4 Å and, as can be seen in Fig. 1, there is a considerable degree of stacking in the structure. The NH . . . O hydrogen bonds between the N6 amino group of adenine and the O4 carbonyl oxygen of uracil (see Fig. 3 for the numbering scheme) have bond lengths of 2.95 Å and 2.91 Å. The NH . . . N hydrogen bonds between uracil N3 and adenine

N1 have lengths of 2.82 Å and 2.86 Å. The important distances across the base pairs between the two ribose carbon atoms C1′ of the glycosidic linkage, are 10.50 Å and 10.53 Å. Similar distances to these have been obtained by analysis of single crystals of intermolecular complexes of purine and pyrimidine derivatives[18] but this is the first time that these distances have been observed in a molecule which forms a fragment of a double helix.

The helical form of the molecules can be seen in Fig. 1 in which one is looking in a direction perpendicular to the base pair plane. There is clearly a rotational relationship between the glycosidic bonds (C1′ to adenine N9 or C1′ to uracil N1) of the front and rear base pairs. Another view of the structure parallel to the stacked hydrogen bonded bases is shown in Fig. 2. The right handed helical rotation can readily be seen by observing the orientations of adjoining ribose residues on the ribose phosphate chain. This structure is similar to that which has been deduced from studying double stranded viral RNA. The naturally occurring material is called RNA 11. Fig. 2 shows similar views of ApU and RNA 11, where the dashed vertical line represents the approximate helix axis. The continued extension of the ApU nucleotide pairs would generate a right handed double stranded helix similar to RNA 11. It is important to note that although the helical parameters of RNA 11 can be derived directly from an X-ray analysis of the fibre, the positions of the atomic centres are of necessity inferred from data derived from single crystal studies of small molecules rather than from direct observation.

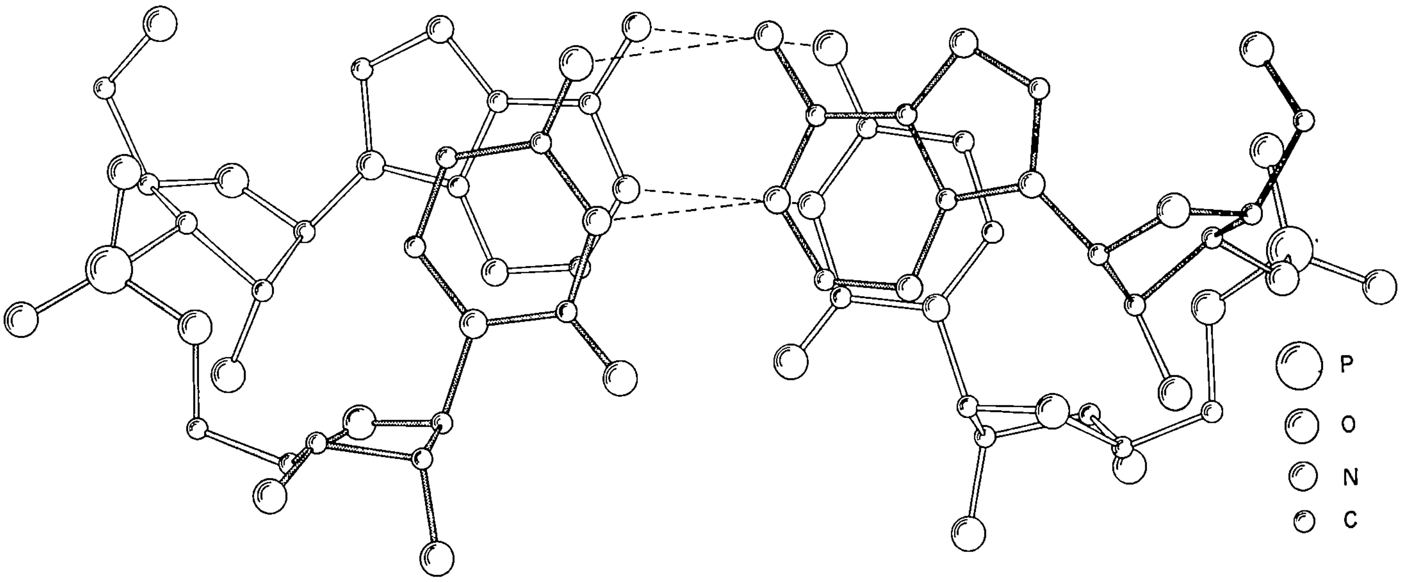

Fig. 1 View of the crystal structure perpendicular to the base planes showing hydrogen bonding (dashed lines) as well as the base stacking interactions. The darkest portions of the figure are those nearest the viewer. The rotational relationship is easily seen by comparing the front (black) and rear (white) glycosidic bonds.

Although the two molecules of ApU in the asymmetric unit are very similar, they are not quite identical. The bond lengths and angles are all within expected range. The conformations of the ribose residues are all 3′-endo[19], and the nucleosides are orientated in the anticonformation[20], with torsion angles about the glycosidic bonds[19] of 2° and 7° for the adenosine residues, and 29° and 30° for the uridines. Comparison with the protonated UpA structure[21,22] suggests that discrepancies between adenine and uracil torsion angles are caused by the differences between the 3′ and 5′ ends of the molecule as the same pattern was obtained with respect to the 3′ and 5′ ends in that molecule which had the opposite base sequence.

In the protonated structure UpA, it was noted that there were short distances between adenosine C8 and O5′, as well as uridine C6 and O5′ (ref. 14). At that time, it was suggested that this might be a result of an intramolecular attraction which, if of a general nature, would help stabilize the anticonformation of the nucleotide. Accordingly, the related distances in this structure were examined with great interest. Three of the four independent distances were greater than or equal to 3.3 Å; thus, there was no evidence of any interaction. But the distance between one adenine C8 and its ribose O5′ was 3.07 Å. As this O5′ is not covalently linked to a phosphorus atom, the phenomenon may not be relevant to the nucleotides in a helical polynucleotide chain. Nevertheless, for non-helical and 3′ terminal nucleic acid structures, this interaction may yet be seen to play an important role.

The individual double helical fragments are separated in the crystal lattice by a spacing of 3.4 Å, with considerable overlapping of the adenine residues. Besides this intermolecular base stacking, the other important interaction in the direction perpendicular to the base pairs involves the proximity of O1′ of both adenosine riboses with uracil rings. A similar type of interaction was found to be important in the crystal structure of UpA[21,22].

The crystal structure can be visualized as a rod-like entity. The central portions of these rods contain the stacked base pairs. These are flanked by the ribose phosphate backbones, both of which in turn are surrounded by the solvent structure. The large amount of water allows the crystal structure to assume a conformation which is minimally perturbed by lattice interactions. Thus, the double helical structure which we observe here bears a marked resemblance to those structures proposed for solvated polynucleotides. It should be noted in passing, however, that the water is held rather firmly, as the intensity data were collected from the crystal while it was mounted in air.

The ribose phosphate chains are packed together with the phosphate groups facing each other. In the layer between the ribose phosphate moieties are sodium ions which are found in two distinct sites: one, complexed between phosphate groups, and the other, surprisingly, bound to the uracil residues. The sodium ions and their ligands are

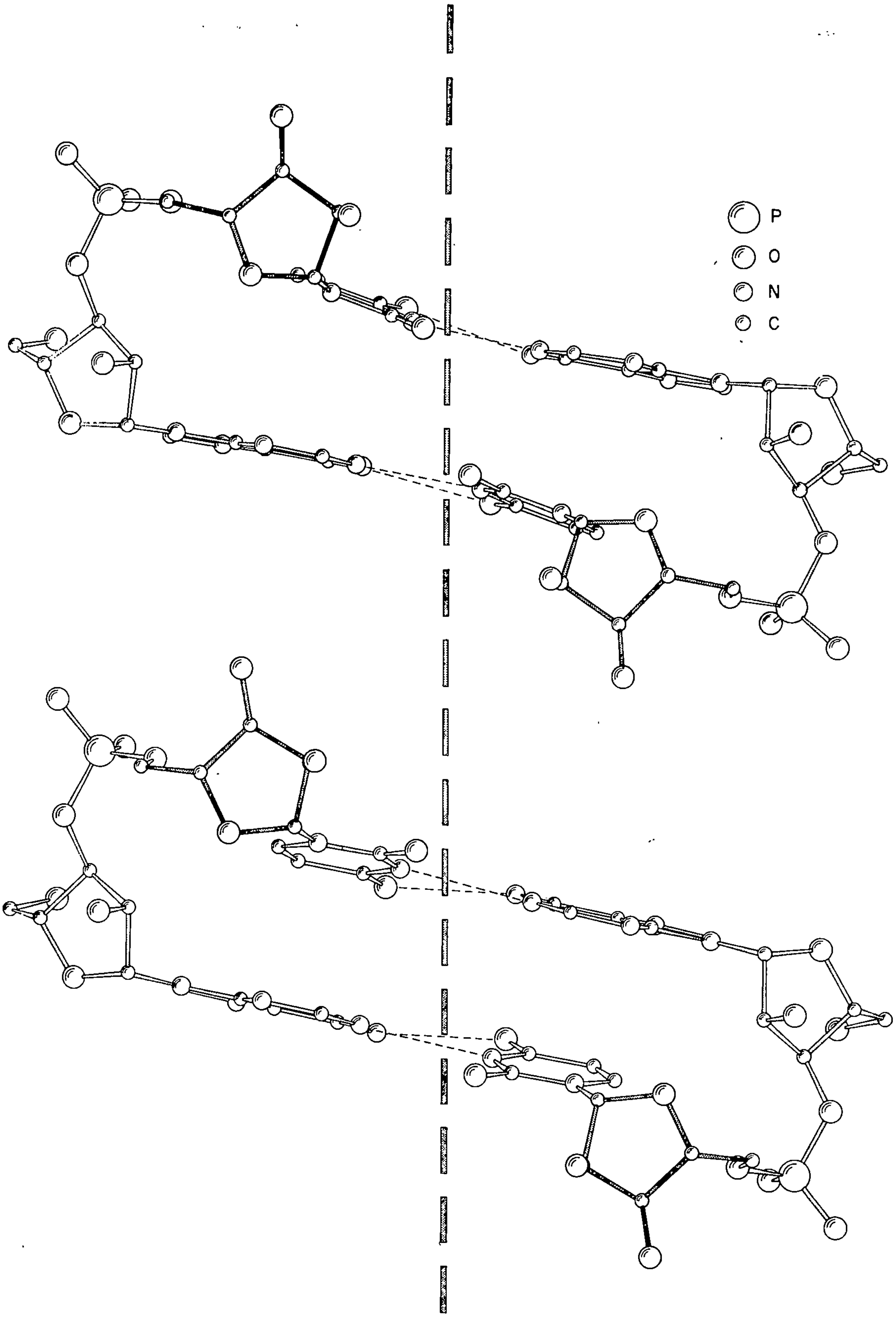

Fig. 2 Comparison of the structures of ApU (upper) and RNA 11 (lower). This view is approximately perpendicular to the helix axis which is indicated by the vertical dashed line.

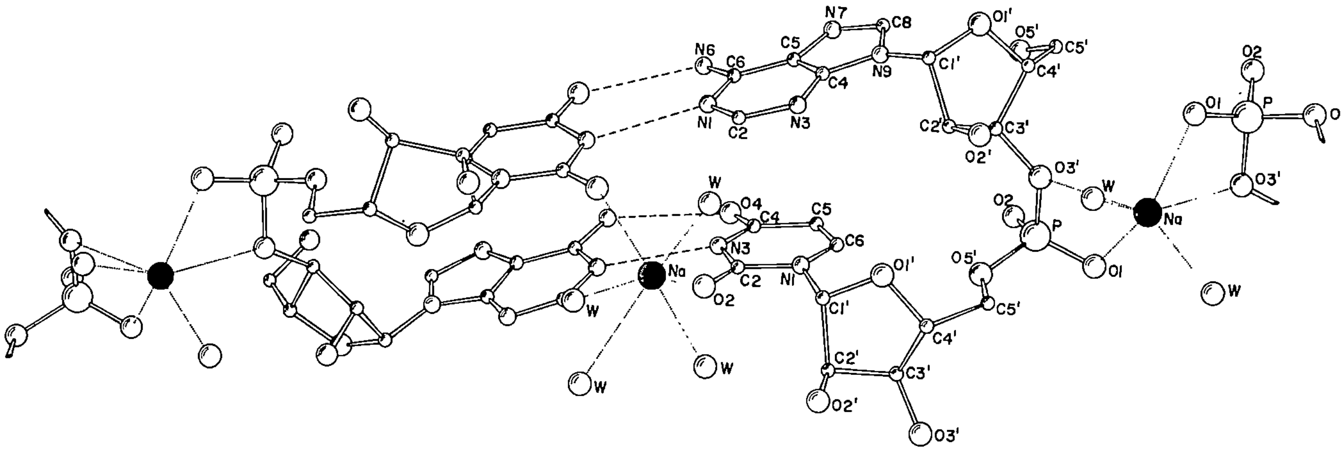

Fig. 3 Perspective view of the structure as seen from the minor groove showing the numbering scheme and the coordination of the sodium ions. Both sodium ions have distorted octahedral coordination involving water molecules (●) and other oxygens. The central sodium ion is 2.36 Å from the uracil O2 atoms.

shown in Fig. 3. The sodium ion in the centre is located on the pseudo two-fold axis in the minor groove of the helix with octahedral coordination which includes the free O2 atoms of the uracils ; the remaining ligands are water molecules. As shown in Fig. 3, the other sodium ion rests on an intermolecular pseudo-dyad axis, also exhibiting octahedral coordination, which includes two adenosyl O3′ atoms and two phosphate oxygen atoms.

The stability of the sodium ion position between two phosphate groups is readily understood on electrostatic grounds. What is not so obvious is why the second sodium ion is complexed to the two uracils in the minor groove of the helix. Five angstroms away from this site there is another position between two phosphate groups which could comfortably accommodate the sodium ion and is occupied only by solvent. It is possible that the structure is stabilized considerably by the sodium ion in the minor groove site. Should this prove to be of more general occurrence in other crystals containing A–U sequences, it is possible that this type of coordination may be of importance in polynucleotide structure and function.

As noted above, one of the most significant features of the organization of the crystal structure of ApU is that the two independent molecules in the asymmetric unit have very similar but not quite identical conformations. Thus, they are related by a pseudo two-fold rotation axis while in an antiparallel double helical polynucleotide there is presumed to be a true two-fold axis which relates the backbones of the two antiparallel chains. We do not completely understand why the crystal structure of ApU did not adopt a true two-fold axis. In this regard, however, we recently discovered that the closely related dinucleoside phosphate, guanosyl-3′,5′-cytidyl phosphate does indeed form a right handed antiparallel double helix in the crystalline state in which this two-fold axis is crystallographic (unpublished results of R. O. Day, N. C. Seeman, J. M. R., and A. R.). This recent observation lends support to our earlier emphasis on the importance of the pseudo two-fold axis in the present crystal structure.

Among the significant facts which we learn by analysing double helical fragments are the important detailed parameters determining the conformation of the polynucleotide chain, especially those parts dealing with the geometry of the phosphate group. The structure of the ribose phosphate chain is of central importance in understanding the physical properties and behaviour of polynucleotide chains[23]. This information should prove valuable in interpreting the details of the molecular structure in polynucleotide double helices, as well as in the more complex forms of RNA such as those observed in tRNA[24].

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LETTERS TO NATURE

PHYSICAL SCIENCES

Anomalous Helium Abundance of the Galactic Centre HII Region G0.5-0.0

We report here a search for the He109α line from the galactic centre HII region G0.5-0.0. Our failure to detect the line sets an upper limit to the abundance of helium which is about 10% of the abundance found in many HII regions. Our limit of 0.01 for the abundance of helium relative to hydrogen is even lower than the limits of 0.021 and 0.025 recently reported[1] for two other galactic centre regions, G0.7-0.0 (Sgr B2) and G0.2-0.0.

Observations of the 109α radio recombination lines of hydrogen and helium were carried out in October 1972 using the Parkes 64-m radio telescope, which has a beam-width of 4.1 arc min at this frequency (5 GHz). The receiving system had an overall noise temperature of 80 K and was followed by a bank of contiguous filters with 100 kHz bandwidth[2] to give an equivalent velocity resolution of 6 km s^{-1}. A digital computer controlled the receiver, switching between the antenna feed and a cooled reference termination, calibrating the system at frequent intervals, and collecting, processing and displaying the data as a sixty-four-point spectrum.

Fig. 1 shows the spectrum of G0.5-0.0 derived by averaging observations totalling 15 h on source. Each observation measured the difference spectrum between the source and a region of blank sky observed over the same zenith and azimuth range. The beam brightness temperature scale was established by observing Hydra A, for which a flux density of 13.50 f.u. was assumed. The velocity scale is for H109α relative to the local standard of rest. The H109α line in G0.5-0.0 consists of an intense component and two low intensity components, indicating at least three HII regions in the line of sight. No significant peak is seen at the frequency of the He109α line.

With the assumption of low optical depth in the continuum and the line, observations of a pair of Hnα and Henα lines yield the ratio of a weighted average of the number of helium atoms in the upper excitation state to the corresponding average for hydrogen atoms in the nebula. This ratio gives the total number abundance of helium to hydrogen using the assumptions that: the helium and hydrogen have the same distribution throughout the nebula; the line enhancement factors and the deviations from LTE populations are the same for both helium and hydrogen; the helium and hydrogen are ionized in the same volume of space; and there is no doubly ionized helium.

With these assumptions; and assuming equal widths for the hydrogen and helium lines (measurement of H109α and He109α lines in twenty southern HII regions yield a mean value for the ratio of the line widths of hydrogen to helium of 1.05±0.16 r.m.s. (unpublished results from Churchwell and Huchtmeier[8] and our work) and an intensity limit of three times the r.m.s. noise, our observations place an upper limit on the number abundance of singly ionized helium (plus heavier elements) of 0.01. This is considerably lower than the previous limit of 0.041 for this source[3].

Our first two assumptions seem to be satisfied in all HII regions and have been discussed in other places (see for example Palmer *et al.*[4]). The other two assumptions are satisfied to varying degrees in different HII regions and will be considered in turn for G0.5-0.0.

For exciting stars of spectral type earlier than about O7, calculations by Rubin[5] have shown that in an HII region of uniform density the Strömgren radii of HeII and HII will be essentially equal. If, however, high density condensations occur within the ionized hydrogen region there will be volumes (shadow zones) where helium is not ionized[6,7]. For Orion A, M8 and M17, in which such condensations occur[8,9] the optical observations of Peimbert and Costero[10] show the abundance of neutral helium to be between 0.01 and 0.03 relative to ionized hydrogen. This is less than one-third of the total helium abundance; if a similar ratio applies for the HII region G0.5-0.0 the presence of condensations will not greatly affect our upper limit. If, on the other hand, the exciting star is of a spectral type later than O9.5 the radius of the HeII region will be considerably smaller than that of the HII region and an appreciable amount of the helium is left unionized. Such a case seems to occur in the HII region NGC 2024 for which the excitation parameter is small (u=27 cm^{-2} pc) and for which the exciting star has been identified as being of spectral type B0.5. For this HII region the upper limit to the singly ionized helium abundance is 0.02 (ref. 4). The present source, G0.5-0.0, has a relatively high excitation parameter (237 cm^{-2} pc; see Table 1) by comparison with other galactic HII regions for which recombination line observations show "normal" helium abundance. Its emission measure, electron density and ionized mass (Table 1) are well within the range of values for other HII regions so it is likely that most of its helium is ionized.

The possibility that an important percentage of the helium is doubly ionized has to be considered. For both Orion A and M17, radio recombination line measurements[4] and optical measurements (Peimbert, see ref. 11) have established an upper limit of 10^{-3} for the number abundance

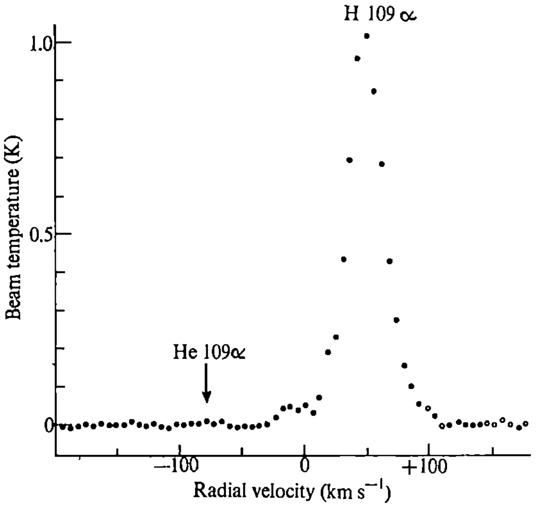

Fig. 1 The H109α recombination line in the galactic centre source G0.5–0.0 is displayed as full-beam brightness temperature against radial velocity of H109α relative to the local standard of rest. The expected frequency of any corresponding He109α line is indicated by an arrow.

Table 1 Measured Parameters of the HII Region G0.5-0.0

Radio continuum flux at 5 GHz	38.3 f.u.
Full-beam brightness temperature:	
Continuum peak	14.2 K
H109α line peak	1.03 K
H109α radial velocity (l.s.r.):	
Principal feature	$+47.4 \pm 1.2$ km s^{-1}
Low intensity features	-13.0 ± 3.0 km s^{-1} $+88.9 \pm 3.0$ km s^{-1}
H109α line width (corrected for filter broadening)	556 ± 20 kHz
N(HeII)/N(HII)	<0.01
Derived parameters *	
Electron temperature (L.T.E. condition), T_e	$5{,}500 \pm 800$ K
Emission measure, E	2.5×10^5 cm^{-6} pc
Electron density, N_e	103 cm^{-3}
Ionized mass, $M_\odot$	1.1×10^4
Excitation parameter, u	237 cm^{-2} pc

* Derived on the assumption of a spherical model HII region.

of doubly ionized helium relative to ionized hydrogen. Martin and Downes[12] found that potential external sources of ionization were too inefficient to ionize helium doubly in G0.7-0.0. It therefore seems reasonable to assume that the amount of doubly ionized helium in G0.5-0.0 is negligible. (For G0.2-0.0 and G0.7-0.0 radio observations[1] established an upper limit for doubly ionized helium of 0.01. Adopting a similar limit for G0.5-0.0 would raise our limit for the singly and doubly ionized helium abundance from 0.01 to 0.02.)

Most astronomical objects, galactic and extra-galactic HII regions, O and B stars, planetary nebulae, solar chromosphere and prominences, and solar cosmic rays have helium abundances between 0.07 to 0.14. Stellar evolution models also require values in this range to match the observed characteristics of stars. Thus from the foregoing discussion it seems that the galactic centre HII regions are deficient in helium—a characteristic they share with many halo Bp stars[13,14] and some QSOs.

Generation of helium within main sequence stars can account for a number abundance of only 0.01 (ref. 15). An alternative hypothesis that helium originated in galactic nuclei ("little bang", see, for example, Burbidge and Burbidge[16]) certainly finds no support from these observations, which show a deficiency near the galactic nucleus. Another possibility is that helium originated in the early stages of the Universe. Independent computations by two groups[17–20] have indicated that a number abundance of about 0.1 could be produced in the universal fireball. On the other hand, a number of authors (see, for example, refs. 20 and 21) have discussed phenomena which might have produced an amount of primordial helium of 0.01 or lower. So far no theory simultaneously accounts for a helium abundance of about 0.1 in most astronomical objects and helium-deficient regions such as G0.5-0.0.

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Physical Background of the Geoidal Figure

R. R. ALLEN has recently considered the depth within the Earth of the origins of the gravitational anomalies, and other studies have been reviewed in *Nature*[1]. Plainly the time has now come when enough is known about the characteristics of the geoid to make possible a definition of density variations within the Earth.

Table 1 Approximation of the Equatorial Section of the Geoid by Two Zonal Spherical Harmonics of Fifth Degree (Σ_1, Σ_2) from the Directions 58° resp. 156.5°

λ (°)	Σ_1 (m)	Σ_2 (m)	$\Sigma_1+\Sigma_2$ computed (m)	Measured (m)	Δ Measured–computed (m)
58	−26.4	−37.3	−63.7	−58.7	+5.0
68	−26.6	−53.5	−80.1	−80.5	−0.4
78	−25.9	−58.4	−84.3	−92.5	−8.2
88	−20.9	−48.5	−69.4	−76.8	−7.4
98	−8.2	−27.6	−35.8	−33.0	+2.8
108	+5.6	−1.4	+4.2	+16.3	+12.1
118	+19.4	+23.6	+43.0	+47.8	+4.8
128	+25.0	+43.2	+68.2	+59.8	−8.4
138	+19.4	+55.4	+74.8	+64.8	−10.0
148	+4.6	+61.4	+66.0	+67.7	+1.7
158	−12.3	+63.0	+50.7	+61.2	+10.5
168	−23.3	+60.0	+36.7	+43.3	+6.6
178	−23.8	+53.2	+29.4	+25.4	−4.0
188	−14.1	+39.0	+24.9	+17.7	−7.2
198	+1.0	+16.8	+17.8	+15.8	−2.0
208	+16.0	−9.2	+6.8	+8.5	+1.7
218	+27.0	−34.3	−7.3	−5.6	+1.7
228	+32.9	−53.2	−20.3	−18.0	+2.3
238	+34.7	−58.7	−24.0	−22.2	+1.8
248	+32.9	−49.8	−16.9	−19.1	−2.2
258	+27.0	−31.3	−4.3	−10.2	−5.9
268	+16.0	−9.7	+6.3	+3.5	−2.8
278	+1.0	+8.4	+9.4	+15.2	+5.8
288	−14.1	+18.7	+4.5	+13.0	+8.5
298	−23.8	+17.3	−6.5	−4.6	+1.9
308	−23.3	+8.7	−14.6	−21.9	−7.3
318	−12.3	−2.3	−14.6	−21.4	−6.8
328	+4.6	−10.7	−6.1	−4.2	+1.9
338	+19.4	−13.5	+5.9	+13.2	+7.3
348	+25.0	−8.5	+16.5	+19.3	+2.8
358	+19.4	+1.2	+20.6	+17.7	−2.9
8	+5.6	+11.7	+17.3	+14.7	−2.6
18	−8.2	+18.5	+10.3	+8.8	−1.5
28	−20.9	+16.5	−4.4	−4.1	+0.3
38	−25.9	+4.5	−21.4	−21.5	−0.1
48	−26.6	−12.8	−39.4	−39.2	+0.2

It has long been known that the eccentricity of the magnetic dipole coincides with the equatorial major axis of the triaxial Earth and that the minor axis of the equator points towards the point of convergence of the magnetic secular variation[2]. I have approximated the equatorial section of the geoid by zonal spherical harmonics in a way that makes use of the axially-symmetric features of zonal spherical harmonics. It emerges that the equatorial section of the geoid can be approximated by the sum of two rigorously symmetrical geometrical forms[3] (Fig. 1).

By varying the direction of the axes of the two symmetrical geometric figures, it is possible to choose a best fit solution; the directions corresponding to this best fit are 58° and 156.5° E.

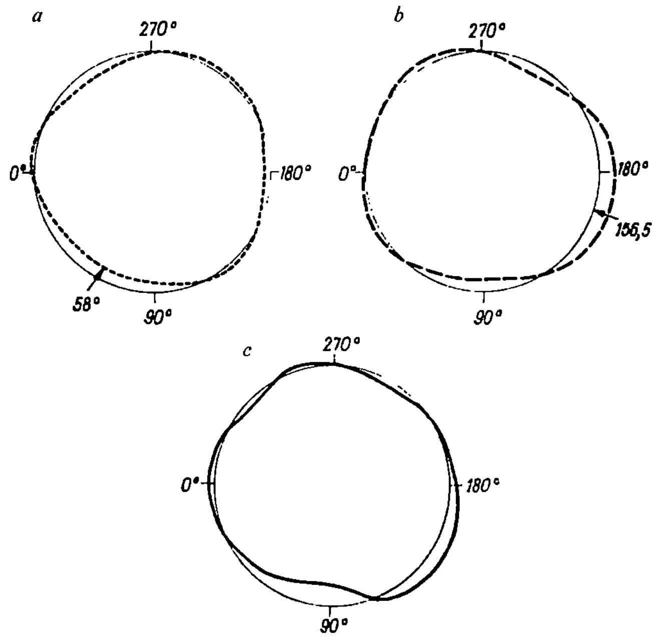

Fig. 1 Approximation of the equatorial section of the geoid by two symmetrical forms from the directions 58° and 156.5° E. *a* and *b*, Symmetrical forms; *c*, their sum.

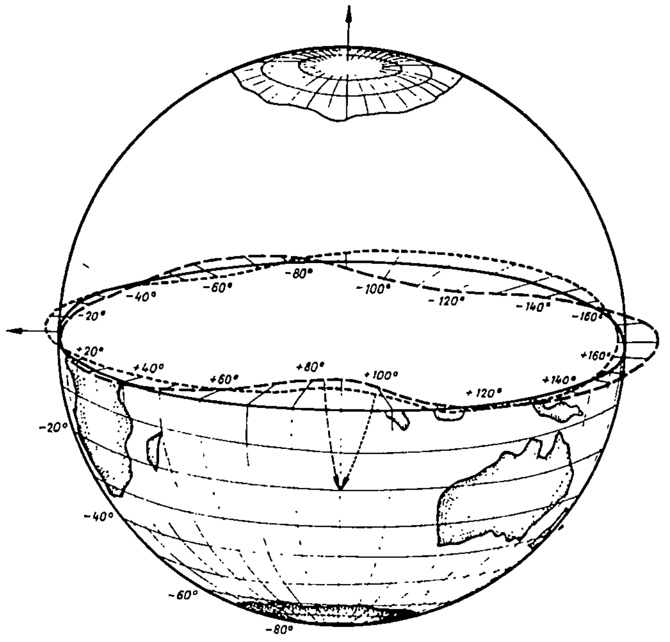

Fig. 2 Computation of the geoid figure from two rotation-symmetrical anomaly systems.

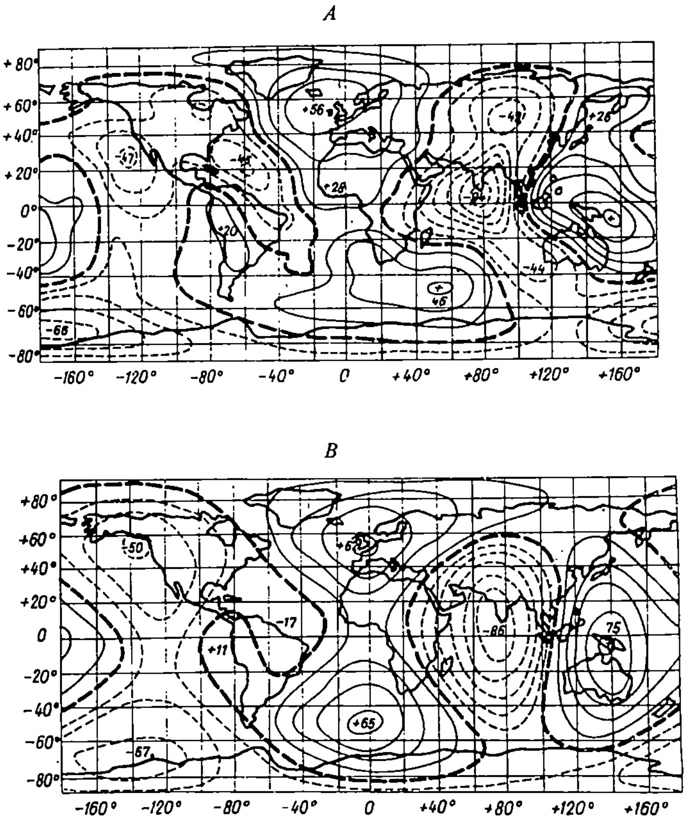

Fig. 3 *A*, The "Smithsonian 1966" geoid; *B*, geoid figure computed from the two rotation-symmetrical anomaly systems. Spacing of isolines, 15 m.

The computations were made in 1969–70 and were based on the equatorial profile of the Smithsonian Standard Earth, 1966. The basic anomalies of the more recent geoids do not differ significantly. The form of these approximating functions is as follows:

$$\Sigma_1 = -0.3 - 3.70\, P_{20} - 46.11\, P_{30} + 8.16\, P_{40} + 15.56\, P_{50}$$

$$\Sigma_2 = -11.8 + 61.74\, P_{20} + 43.50\, P_{30} - 25.13\, P_{40} - 5.10\, P_{50},$$

where P_{i0} are the zonal spherical functions related to the point 58° E of the equator as polar point, or for the point 156.5° E. The positions of the symmetry points and the form of the functions refer to the geoid under consideration only, but a more general validity may be attributed to them because of the similarity of geoid figures obtained in different ways.

Substituting into the zonal harmonics the angle of distance of the single points from the corresponding polar point gives the single symmetrical anomaly components of the equatorial section, the sum of which closely approximates the actual section (Table 1).

Assuming the rotational symmetry of the anomalies thus obtained in the equatorial plane and computing the spherical distance measured from the approximating directions for the grid of points having integer coordinates, one easily obtains a map-like distribution of the anomaly components sum over the globe's surface (Fig. 2). By eliminating the polar flattening in the course of summation and by taking into account the well known pear-shape of the Earth, a theoretical geoid figure is obtained (Fig. 3).

The geoid figure thus computed is similar to and even identical with the measured figure as regards both the position and the size of anomalies.

It is important that I have used for the construction of the computed geoid merely the equatorial data system of measurements. The identity of these two figures cannot therefore be regarded simply as a result of approximation, but it can be considered as a proof of the fact that the complex geoid figure is merely the sum of two rotation-symmetrical anomaly systems and no other agents play an important role in the formation of the geoid pattern.

Certain further conclusions may also be drawn from the coefficients of the approximating functions. The zonal harmonic series connected with the direction towards Australia converges uniformly and the coefficients of the successive terms of even and odd degree are regularly decreasing. On the other hand, the even terms of the function in connexion with India are small compared with those of odd degree, that is, this form shows no ellipticity.

The coefficients of the mathematical formulae and the figure that can be computed with them support the hypothesis that—according to the eccentricity of the magnetic field—a deep-lying positive inhomogeneity of material character exists in the direction of Australia; nearly perpendicular to this, near the symmetry-point of the secular magnetic variation, a similarly essential geoid deformation exists, associated, however, with energy effects.

Besides the high degree of similarity, the theoretical and the measured geoid patterns also show certain characteristic differences. The axes of the positive and negative anomaly systems of the northern, resp. southern hemispheres—measured on the map—are not perpendicular to the line of the Equator. Consequently, when determining the position of the responsible density inhomogeneity, one has to step out of the Equator. By that means, a still better approximation than the present would be obtained.

From the foregoing it is clear that the geoid is the result of two effects originating at great depth and in all probability is not linked with the distribution of oceans and continents on the surface of the globe. The starting point of this study is the coincidence of the direction of eccentricity of the magnetic dipole with the great positive geoid anomaly over Australia. If the two phenomena are causally related, one must suppose the existence of a secular variation of the gravity field as well as of the geoid.

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Yellowstone Convection Plume and Break-up of the Western United States

MUCH of the late Cainozoic history of the western United States is characterized by tensional rifting. The Basin and Range Province is a zone of rifting 300 to 600 km wide (Fig. 1). The Rio Grande depression and the Snake River downwarp are areas of rifting which are similar in time and style to the Basin and Range faulting. The rifting in the Rio Grande depression has usually been related to the same causative mechanism as in the Basin and Range. The Basin and Range type faulting has variously been explained as the result of a westward moving convection cell[1]; as part of a wide, soft boundary reflecting the transform motion of the American and Pacific plates[2]; or as the result of a large mantle diapir which came up under the crust following the complete subduction of the Farallon plate in that region[3]. Although most of these explanations adequately account for the Basin and Range faulting, they fall short in explaining the rifting in the Rio Grande depression. None of the three hypotheses adequately explains why extensive rifting occurs over a area 600 km wide in the Basin and Range abruptly stops for a length of 500 km in the Colorado Plateau area and then resumes again in a narrow zone in the Rio Grande region. The Snake River rifting transects the regional trend of the Basin and Range rifting and does not fit any of the proposed tectonic models for the Basin and Range.

We propose that the mechanism of rifting in the Rio Grande depression and Snake River downwarp is different from the mechanism of rifting in the Basin and Range region. The rifting in the Rio Grande region of the Southern Rocky Mountains and in the Snake River Plain is the result of the break-up of the western United States by the Yellowstone convection plume, whereas the Basin and Range faulting may be a result of one of the three previously mentioned hypotheses.

The Snake River downwarp (Fig. 1) is an arcuate graben more than 500 km long that has formed since early Pliocene[4]. The depression is filled with continental detrital sediments and tholeiitic basalt flows[4]. Thickness of the fill is estimated to range from 100 to 1,500 m in the eastern part of the depression[5] and may exceed 3,000 m in the western part[6].

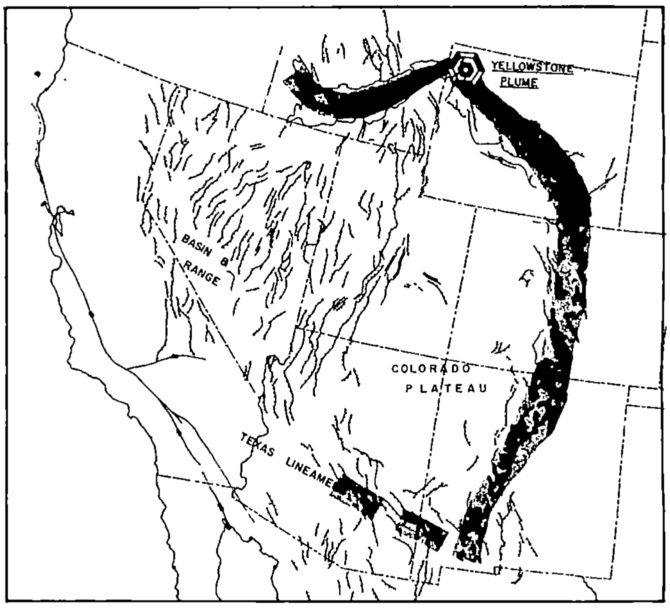

Fig. 1 Index map of western United States showing normal and reverse faulting, location of the Yellowstone convection plume, and the approximate boundaries (shaded areas) of newly forming plate. (Low-angle thrust faults are not included on this map.)

The Rio Grande depression is a late Cainozoic feature which extends from southern New Mexico 800 km into central Colorado (Fig. 1). It is a complexly faulted graben which may contain as much as 10,000 m of fill[7]. Basalts with alkalic affinities and those with tholeiitic affinities are spatially related to the depression in the same manner as the tholeiitic basalts and alkali olivine basalts are spatially related to the East African rift valley[8].

The volcanic activity in the Yellowstone region has been attributed to a mantle convection plume located under that area[9,10]. Interpretations of absolute motions of lithosphere plates indicate that North America has been moving very slowly over the Yellowstone plume for the past 40 m.y. (refs. 10 and 11). A lithosphere plate which is stationary or moving slowly over a mantle convection plume presumably will break up into two or more smaller plates. A convection plume in the vicinity of the Afar triangle seems to be causing the rifting in the Red Sea, Gulf of Aden, and East African rift valley. We propose that the Yellowstone plume is causing the break-up of the American plate in a somewhat analogous manner. Most of the western United States is part of a new plate which is trying to break free from the rest of the American plate.

Boundaries of the newly forming plate are the Snake River downwarp, Middle and Southern Rocky Mountains, Rio

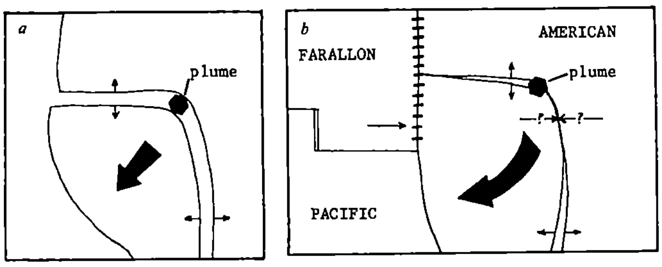

Fig. 2 *a*, Example of ideal case where rifting and break-up of lithosphere results from slow movement over a convection plume; *b*, rifting that might result from break-up of western United States by Yellowstone plume. Trench boundary along northern part of west coast during the late Miocene would act as a barrier to movement, while the southern part would be free to move. The resulting motion would be a somewhat clockwise rotation of the new plate.

Grande depression, and possibly the Texas Lineament (Fig. 1). Under ideal circumstances, a plume causing the break up of a plate would propagate cracks away from the plume with subsequent rifting as illustrated in Fig. 2*a*. Although the Snake River downwarp extends outward from the plume, the Rio Grande depression begins some 800 km from the plume. Obviously this distribution of rifting does not resemble the ideal case. But the late Cainozoic plate tectonic history of the west coast of the United States provides a possible explanation for the distribution of rifting and uplift around the margins of the new plate.

Although the American plate has been moving very slowly over the Yellowstone plume for the past 40 m.y., the plume may not have been effective in breaking up the plate until more recently because of the interaction of the Farallon plate with the American plate during the late Cainozoic. Before 29 m.y. ago a trench boundary between the American plate and the Farallon plate existed off the entire coast of the western United States. Underthrusting by the Farallon plate probably acted as a barrier to the westward movement of the newly forming plate. The trench boundary changed to a transform fault boundary when the Pacific plate came in contact with the American plate. Therefore, once the trench was shut off, the new plate was able to break free. But the entire trench was not shut off at the same time. It first disappeared off northern Baja California[12]. Elimination of the trench then progressed from Baja California northward to its present location off Cape Medicino[12]. This set the southern part of the new plate free to rift while still acting as a barrier to the northern part. The resulting motion would be a clockwise rotation away from the Yellowstone plume as depicted in Fig. 2*b*.

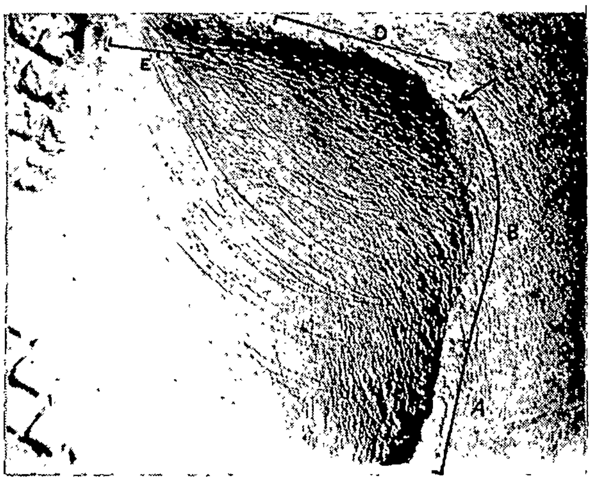

Fig. 3 Clay cake simulation of movement of new plate. Rigid wire mesh was placed partially under clay cake with apex located at *C*. The rigid wire mesh was given a combination of tension and clockwise rotation away from *C*. A large rift (0.5 cm) developed in region *A* similar to the Rio Grande depression. Minor breaking occurred in area *B* which is analogous to middle and southern Rocky Mountains. In nature, this minor breaking would cause isostatic adjustment in the mantle and uplift of the Rockies in the late Cainozoic. The Yellowstone plume is represented by *C*. Area *D* is another significant rift (0.4 cm) which corresponds to the Snake River downwarp. Area *E* is an area of little or no faulting.

To test this idea, we performed clay cake experiments in which a rigid plate under the clay cake, with its apex representing the Yellowstone plume, was given this type of rotational movement. Fig. 3 shows that the resulting distribution of the rifting is nearly identical to that in the western United States. A large rift developed near the apex (Yellowstone plume), similar to the Snake River downwarp. Another large rift developed at some distance from the apex, similar to the Rio Grande rift which developed at some distance from the Yellowstone plume. Late Cainozoic uplift in the Middle and Southern Rocky Mountains can be attributed either to compression resulting from the rotation or to uplift prior to major rifting.

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Dislocation Vibration as a Possible Cure for High Temperature Void Formation in Metals under Fast Neutron Irradiation

EXTENSIVE void formation occurs in metals subjected to fast neutron irradiation in the temperature range of 0.3 T_m to 0.5 T_m where T_m is the melting temperature of the metal[1-4]. This phenomenon is a serious problem in reactor technology. In conditions of fast neutron irradiation large numbers of lattice vacancies and interstitial atoms are produced in equal amounts. Dislocation lines and voids (pre-existing ones or newly nucleated ones) act as sinks to these point defects. If a void is just as efficient a sink for an interstitial atom as for a lattice vacancy and if a dislocation line also is just as efficient a sink for each of these types of defect there can be no tendency for a void to grow[2-4]. Equal numbers of vacancies and interstitials will be destroyed in a void as well as at dislocation lines. But dislocation lines are probably a more efficient sink for interstitials than for vacancies. Thus more interstitials are destroyed at dislocation lines than are vacancies. The excess vacancies are destroyed at voids, causing them to grow. This very plausible explanation has been presented in the literature to account for growth of voids (see refs. 2–4 and the references cited there).

If a dislocation can be made to be as efficient (or more efficient) a sink for vacancies as for interstitials, void growth will be prevented. Here we point out how a dislocation can be made a better sink for vacancies.

The theory that Ham[5] developed for impurity segregation to a dislocation line has been applied[2] to show that in general an edge dislocation is a better sink for interstitials than for vacancies. If an edge dislocation line has no stress field associated with it diffusion of point defects to it is equivalent to diffusion in a cylinder of inner radius b, where b is the Burgers vector of the dislocation, and outer radius R, where R is half the mean spacing between dislocations. The defect concentration at the inner radius is the equilibrium concentration appropriate to the temperature of the metal. The concentration at the outer radius is greater than the equilibrium concentration because of the defects produced by the neutron irradiation. An edge dislocation does, however, have a stress field and vacancies and interstitials will interact with this field. Ham showed how this interaction affects the diffusion around a dislocation. The effect of the interaction is to increase the "effective" inner radius of the cylinder from b to a value that increases with the strength of the interaction. The larger the effective value of the inner radius, the better sink is the dislocation line. Because interstitials interact more strongly with the stress field of an edge dislocation than do lattice vacancies, the dislocation is a better sink for interstitials than for vacancies. The calculations of Harkness and Li[2] seem to indicate that the effective inner radius for interstitials is increased by about $2b$. (Bullough and Perrin[3] concluded that to make a fit to experimental data a dislocation line is a 1 to 2% better sink for interstitials than for vacancies.)

If a dislocation which is acting as a sink for point defects is set into oscillatory motion of frequency ν and amplitude λ where $\lambda > b$, then in a time $t = 1/\nu$ the characteristic diffusion distance d for the point defects is approximately given by $d \sim \sqrt{(D/\nu)}$, where D is the diffusion coefficient of either the vacancies or of the interstitials. If $d \gg \lambda$ the diffusion takes place at such a fast rate that for all practical purposes the dislocation can be considered to be a stationary sink for the point defects. But if $d \sim \lambda$ or if $d < \lambda$ the dislocation moves back and forth quickly enough so that it can be considered to be a sink for point defects with a dimension on the slip plane (or an "effective" inner radius) of λ rather than b. Clearly in this situation the dislocation is a more efficient sink than when it is not vibrating.

Interstitial atoms in metals diffuse with such high velocities in the temperature range in which void formation is extensive that it is very difficult to make a dislocation a better sink for interstitials by vibrating the dislocation. For example, stainless steel[2] forms voids in the temperature range 300° to 600° C. The diffusion coefficient D for interstitials in this temperature region calculated using the activation energies for diffusion estimated by Harkness and Li[2] or by Bullough and Perrin[3] is of the order of 1 to 10 mm^2 s^{-1}. For frequencies of 1 Hz to 1 kHz the diffusion distance d is in the range 0.03 to 3 mm. It would be impractical to vibrate dislocations with an amplitude comparable to these values of d.

Vacancies in metals diffuse much more slowly than do interstitial atoms. For stainless steel in the temperature range of 300 to 600° C the estimated value of D (refs. 2 and 3) is of the order of 6×10^{-7} to 3×10^{-11} mm^2 s^{-1}. At frequencies of 1 Hz to 1 kHz the diffusion distance d is between 2×10^{-7} mm and 8×10^{-4} mm. Certainly it is practical using moderate oscillating stresses to force dislocations to vibrate with amplitudes in the lower part of this range, say, 2×10^{-7} mm to 2×10^{-5} mm. Thus it seems that by forcing them to vibrate it is possible to make dislocations better sinks for vacancies. It may be possible, therefore, to eliminate void formation, at least in the lower part of the temperature range in which voids form through forcing dislocations to vibrate. The technique of vibrating the dislocations under cycle stresses can be used to obtain new experimental information on the relative efficiency of dislocations as sinks for interstitials and for vacancies.

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Field Observations of Edge Waves

Edge waves are solutions for wave motion trapped against a shoaling beach. Their amplitude varies sinusoidally along the shore and diminishes rapidly seawards from the shoreline. Eckart[1] has shown that there is a family of edge wave solutions of the shallow water equations. Each solution is characterized by a mode number which gives the number of zero crossings in the seaward decay of amplitude; each mode has a dispersion relation linking the longshore wavenumber with the edge wave frequency.

Edge waves have been observed in the laboratory; Bowen and Inman[2] showed that waves of a single frequency ω approaching the shore can excite edge waves close to the same frequency or at the subharmonic frequency $\omega/2$. The existence of edge waves on natural shorelines has been inferred from the periodic spacing of rip currents[2] and the existence of beach cusps and crescentic bars[3] but no previous direct measurements of the water motion associated with edge waves on natural beaches have been reported. Here we present preliminary results, from field measurements of water currents close to the shore, which strongly suggest the existence of a subharmonic, zero order edge wave.

The measurements were made during August 1972 at Slapton Beach, South Devon. This is a shingle ridge running in a gentle curve for more than 5 km. Surveys showed that the beach profile in the inter-tidal zone could be approximated by the expression

$$h = h_0(1 - e^{-ax}) \qquad (1)$$

where h is the water depth at high tide, x the offshore coordinate and for Slapton Beach $h_0 = 7.05$ m and $a = 3.14 \times 10^{-2}$ m^{-1}.

The onshore and longshore horizontal components of the water current were measured by an electromagnetic flowmeter[4] held 0.3 m above the seafloor on an aluminium base. The flowmeter was placed in a fixed position in the intertidal zone so that with the incoming and receding tide its distance from the shoreline varied from 0 m to 11 m and back to 0 m within a 4 h period spanning the time of high water. During this period current meter readings were logged digitally every 0.5 s for separate runs of duration of 15 min.

Spectral analyses of four pairs of fifteen minute time series obtained on August 10, 1972, have been made using the fast Fourier transform technique and a smoothing filter[5]; a typical spectrum of current amplitude is shown in Fig. 1. The fast rise in the spectrum to a broad peak followed by a steady decay with increasing frequency is typical of a wind wave spectrum, and the broad peak occurs at around 0.2 Hz, corresponding to the observed breaker frequency in the surf zone. An unusual feature of the spectrum, however, is the rather sharp peak at about 0.1 Hz, about one half of the predominant wind wave frequency. This energy peak occurs in each of the spectra and for both onshore and longshore components of the flow.

The variation with offshore distance of this energy at 0.1 Hz suggests that it is due to the presence of an edge wave. In Fig. 2 the triangles represent the measured r.m.s. amplitudes at 0.10 ± 0.02 Hz plotted as a function of the offshore position of the current meter, for onshore (Fig. 2*a*) and longshore (Fig. 2*b*) components of the flow. Because the breakpoint occurred at about 6 m from the shoreline it is clear that the amplitude continues to increase well into the surf zone. In contrast, other peaks, including the wind wave peak at 0.2 Hz, showed a slight rise towards the breakpoint and then became indistinguishable inside the surf zone. Any edge wave at the incident wave frequency itself, if present at all, was of very small amplitude.

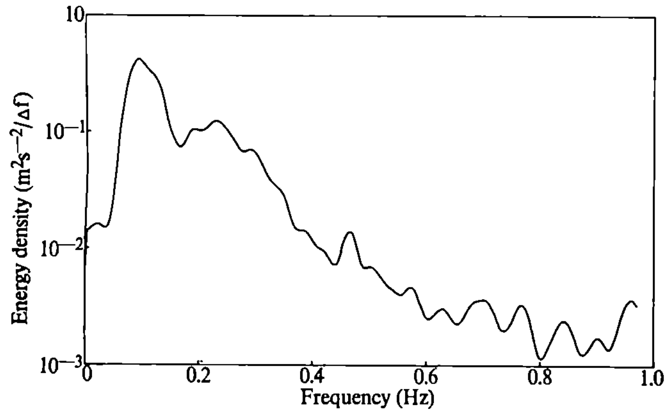

Fig. 1 A typical onshore current spectrum, showing the wind wave peak at 0.2 Hz and the additional peak at 0.1 Hz. The offshore distance was 9.0 m. $N=1{,}536$; $\Delta t=0.5$ s; $\Delta f=0.025$ Hz.

It is not possible to explain the observed amplitudes at 0.1 Hz in terms of a standing gravity wave formed by reflexion at the shoreline, because that would lead to quadrature of the longshore and onshore current components, whereas the observed components are found to be in phase with each other.

The ratio of onshore to longshore current amplitudes is approximately constant, independent of offshore position, at 4.6 ± 1.0. This is consistent only with the $n=0$ edge wave mode and would be inconsistent with any higher order mode. Because the value of the ratio is not unity, as would be expected for a progressive edge wave, the currents must be associated with a trapped standing edge wave. This is confirmed by the observed phase relationship between the horizontal current components because a progressive edge wave implies phase quadrature whereas a standing edge wave gives the observed in-phase relationship. For a standing edge wave, the observed amplitude ratio shows that the current measurements must have been made about 1.3 m along the shore from a longshore current node.

Using the dispersion relations derived by Ball[6] for a beach with a profile given by equation 1, we have calculated the predicted wavelengths of 0.1 Hz edge waves for Slapton Beach, and find for the zero mode a wavelength of 32 m. Ball's theory shows that the zero mode edge wave decays exponentially with distance from the shoreline, with a decay constant dependent on the edge wave wavelength L and the slope parameter a.

Accordingly we have plotted (Fig. 2) the exponential decay corresponding to an edge wave of wavelength 32 m. The measured values fit the expected edge wave curve to within the statistical uncertainty. The least squares best fit of the data to an exponential curve gives $L=34\pm5$ m for the onshore current and $L=34\pm6$ m for the longshore current.

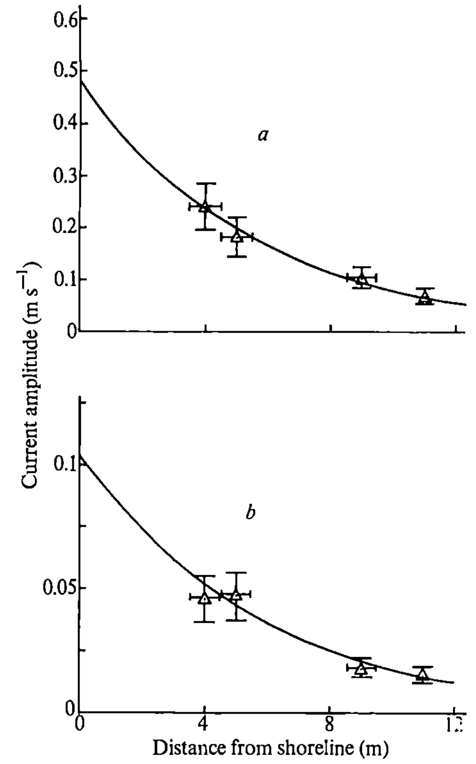

Fig. 2 Graphs of onshore (*a*) and longshore (*b*) current amplitude at 0.1 Hz plotted against distance from shoreline. The solid line shows the expected decay for an edge wave of the predicted wavelength of 32.0 m. The measured values are shown as triangles with amplitude error bars corresponding to the 80% confidence limits in the spectral analysis; horizontal errors are a consequence of averaging amplitudes during an incoming tide.

The longshore variation in horizontal excursion of water up the beach expected from the observed edge wave would have been too small (horizontal amplitude ≃0.5 m) to be observed above the general irregularity caused by the incoming waves. Any tendency of the edge wave to form beach cusps may have been overriden by the oblique angle of approach of the waves which was about 10° from normal at the breaker line. In fact there were no obvious variations in longshore topography which could have induced the observed nearshore motion[7].

These results from a fairly steep beach with small incident waves may have important implications for the mechanism of edge wave generation. Bowen and Inman[2] remark on an apparent correlation in their laboratory experiments between the amplitude of edge waves and the magnitude of the standing wave caused by reflexion of the incident wave at the beach. Trapping of the edge wave may seem rather unexpected because the edge wavelength of 32 m is small in comparison to the length of the beach, which extended about 2.5 km on either side of the point where measurements were made. But standing edge waves are also suggested by crescentic bars which are known to occur on long straight beaches as well as in confined bays[3]. The mechanism responsible for generating edge waves may favour the formation of standing rather than progressive edge waves.

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BIOLOGICAL SCIENCES

Oncogenic Transformation of Hamster Embryo Cells *in vitro* by Adenovirus Type 5

JUDGED on the basis of virus inoculation tests in newborn hamsters[1], human adenovirus type 5 is considered to be non-oncogenic. The virus does transform rat embryo cells with low efficiency *in vitro*[2,3], although these cells do not form tumours in rats (my unpublished results). Infection of hamster cells *in vitro* with type 5 adenovirus gives rise to a productive infection (ref. 4 and my unpublished results) and most of the cells are killed. Because of this extensive cell killing transformation of hamster cells by adenovirus type 5—if it does occur—is unlikely to have been detected. To determine if adenovirus type 5 can transform permissive cells, hamster embryo fibroblasts (HEF) were infected with temperature sensitive (ts) mutants[5] at the restrictive temperature which prevents cell killing caused by virus replication. The mutants used transform non-permissive rat embryo cells at the restrictive temperature (my unpublished results) with frequencies similar to wild type virus, thus the viral function(s) involved in transformation of those cells are not affected by these particular *ts* mutations.

In the work described here, secondary cultures of HEF were infected with one of the *ts* mutants *ts1*, *ts4* or *ts14* at an input multiplicity of 10 plaque-forming units (PFU) per cell. For comparison some cultures were also infected with a similar concentration of wild type virus. For transformation the infected cells (and control non-infected cells) were incubated at 38.5° C for 2 days, then subcultured by seeding at a density of either 4×10^5 or 10^6 cells per 50 mm dish. These subcultures were then cultivated at 38° C for up to 6 weeks. Eagle's medium lacking calcium[2] and supplemented with 5% calf serum was used for maintenance, and the culture medium was changed every 3 to 4 days.

For measurement of virus replication at 38.5° C and at 31° C some cultures were collected at 40 h and at 120 h after infection respectively. Table 1 shows the virus yields in HEF cells at both temperatures. Whereas wild type virus grows equally well at both 38.5° C and 31° C, the mutants grow almost like wild type virus at 31° C but show little or no growth at 38.5° C as was previously found for the mutants in HeLa cells[5]. Although not shown here, there was less efficient growth of adenovirus type 5 in HEF cells than in HeLa cells; nevertheless the yields represent almost a four log increase over the amount of infectious cell-associated virus detected immediately after adsorption.

In the cultures infected by the *ts* mutants and maintained at 38° C, transformed foci first appeared approximately 4 weeks after infection. After a further week the foci could be clearly distinguished from the non-transformed cell background, and at this time foci were counted. The morphology of these transformed HEF cells (Fig. 1) was similar to that of rat embryo cells transformed by adenovirus type 5 (ref. 3). The results of one complete experiment are shown in Table 2. The transformation frequencies observed on infection with the *ts* mutants are slightly lower than those obtained on rat cells with either wild type virus or with these *ts* mutants (my unpublished results).

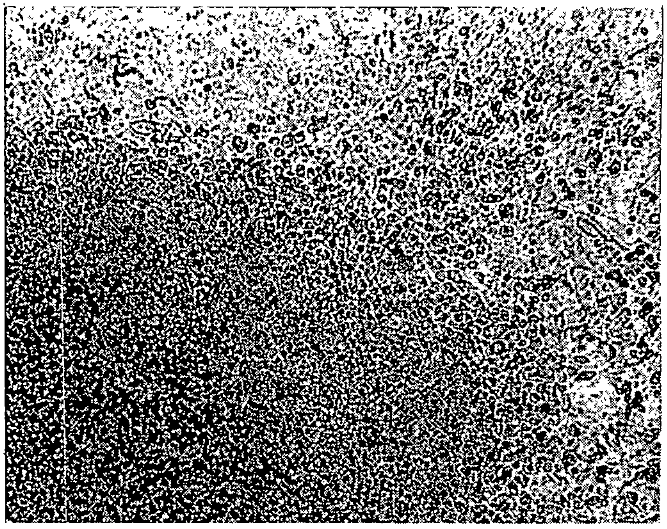

Fig. 1 Focus of HEF cells transformed by adenovirus type 5. Cells infected 30 days previously. Unstained. ×60.

That the same number of foci are produced on dishes seeded with 4×10^5 cells and dishes seeded with 10^6 cells suggests that focus formation may be partly inhibited by non-transformed cells, which rapidly grow to confluence when seeded at higher density.

In this experiment the plating efficiency of the cells infected by wild type virus was reduced to approximately 13% of the plating efficiency of the non-infected controls, and the size of the colonies formed by the survivors was reduced. The plating efficiency of the cells infected by the mutants was also reduced, but to a much smaller extent. Although colonies grew on the dishes seeded with low numbers of wild type infected cells, no cells survived to form colonies on dishes seeded at the higher densities of the transformation assay. The reason for this effect is not established, but it may be due to secondary infection of non-infected cells by contact with primarily infected cells at the higher cell densities.

A number of single foci were picked from cultures infected by each mutant and six lines were established from these. Cells of all six lines retained the transformed morphology throughout many passages *in vitro*. Cells of the lines did not produce virus spontaneously at 38° C, nor were they induced to do so when cultivated at 31° C, the permissive temperature for the *ts* mutants used to transform the cells. The transformed cells could be superinfected by wild type virus (Table 3) at either

Table 1 Yields of Adenovirus Type 5 and *ts* Mutants in HEF Cells.

Virus	38.5° C	31° C
Wild type	1.2×10^8*	9.0×10^7
ts1	2.0×10^3	4.0×10^7
ts4	1.6×10^4	1.2×10^8
ts14	3.2×10^3	1.0×10^7

Monolayers of HEF cells on 50 mm plastic dishes were infected at an input multiplicity of 10 PFU per cell. Infected cells were collected after 40 h at 38.5° C or after 120 h at 31° C.

*, PFU per dish.

Table 2 Transformation at 38° C of HEF Cells by *ts* Mutants of Adenovirus Type 5.

Virus	No. of foci per dish		Plating* efficiency
	4×10^5 cells	10^6 cells	%
Wild type	0, 0, 0	0, 0, 0, 0, 0	0.5†
ts1	2, 2, 3	1, 2, 1, 2, 2	1.7
ts4	2, 2, 1	1, 1, 2	2.1
ts14	2, 2, 1	2, 1, 2, 1, 0	3.2
None	0, 0, 0	0, 0, 0, 0, 0	3.8

*, 10^3 cells plated per dish; †, small colonies.

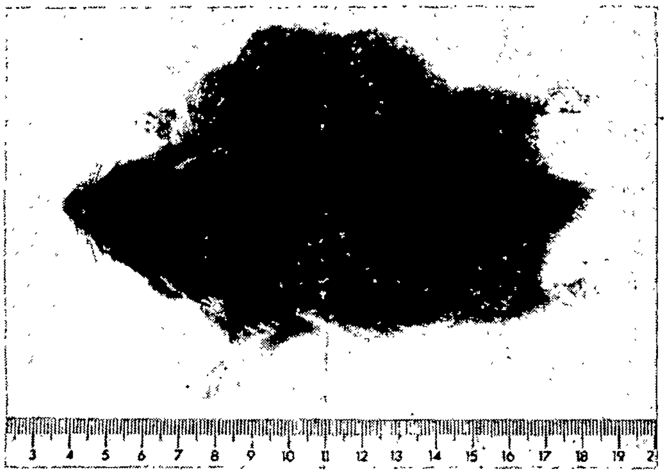

Fig. 2 Subcutaneous tumour in hamster resulting from cells inoculated 60 days previously.

temperature and by various *ts* mutants at the permissive temperature of 31° C, although with the mutants used the ensuing yields were lower than the yields attained in non-transformed hamster embryo cells. The capacity for superinfection presents the possibility of detecting virus genes in these cells either by complementation of *ts* mutants at the non-permissive temperature or by production of recombinants at the permissive temperature. So far the results with a few mutants provide no evidence for either interaction in the two transformed lines tested, but the problem is being further investigated using other mutants, and other transformed lines.

Ability to superinfect makes it at least theoretically possible that host range mutants of the type obtained for polyoma[6] could be obtained. Clearly, these hamster cells transformed by adenovirus type 5 differ from those transformed by adenovirus type 12 (ref. 6), in which superinfection by adenovirus type 2 was not possible. The results suggest the absence of effective repressor or inhibitor in these cells that have been transformed by type 5. We have no evidence that viral genes persist and are active in these cells, but experiments designed to determine if this is the case are in progress.

The cells of two of these lines designated HT5 14a and HT5 14b have been tested for tumorigenicity by inoculation into hamsters. After five passages *in vitro* cells of each of these two lines were inoculated intraperitoneally into 1-day-old hamsters. Approximately 6 weeks later, some of these animals had developed intraperitoneal tumours (Table 4). About 8 weeks after inoculation the tumour-bearing animals were killed, the tumours explanted and grown *in vitro* for two to four passages before reinoculation into 3-day-old baby hamsters. The cells which grew from the tumour explants *in vitro* were morphologically identical to input transformed cells, and they grew rapidly to form tumours when reinoculated into 3-day-old hamsters (Fig. 2). These tumours are transplantable and have been transplanted for up to six consecutive passages. At later passages tumours were formed in 2-week-old recipients, and in adult hamsters. In a few animals metastases to either lungs or liver have been observed. Non-infected, non-transformed hamster cells passaged *in vitro* fifteen times failed to develop tumours in 1-day-old hamsters, suggesting that the oncogenicity of the adenovirus-transformed cells was not spontaneously acquired. The results of some of the transplantation tests are shown in Table 4.

Table 3 Growth of Adenovirus Type 5 in HEF and Transformed HEF (HT) Cells at 38.5° C.

Experiment	Cells	Virus yield 0 h†	Virus yield 40 h
1	HEF	8.5×10^4	2.3×10^8*
1	HT5 14b	3.8×10^4	4.4×10^8
2	HT5 1a	4.4×10^3	3.0×10^7
2	HT5 4a	3.0×10^3	2.0×10^7

Monolayers were infected at an input multiplicity of 10 PFU per cell. *, PFU per dish; †, immediately after absorption.

Table 4 Tumour Formation in Baby Hamsters with Embryo Cells Transformed *in vitro* by Adenovirus Type 5

Cells	Passage number *in vitro*	Age of recipients (days)	Route of inoculation	No. animals with tumours/ No. inoculated
Non-transformed HEF	15	1	s.c.*	0/11
HT5 14a	5	1	i.p.†	2/8
HT5 14b	5	1	i.p.	2/8
HT5 14a	14	3	s.c.	5/6
	Transplant number			
HT5 14a tumour	2	3	s.c.	5/5
HT5 14a tumour	3	3	s.c.	7/8
HT5 14a tumour	4	3	i.p.	4/5
HT5 14b tumour	2	3	s.c.	3/3
HT5 14b tumour	3	14	s.c.	6/7
HT5 14b tumour	4	6	s.c.	5/5

* S.c., Subcutaneous; † i.p., intraperitoneal.
2×10^6 cells inoculated per animal.

The histology of the tumours has been examined by Dr J. M. Vetters of the Pathology Department, Glasgow University. They appear to be largely of the small cell sarcoma type or possibly malignant lymphoid neoplasms with necrotic centres typical of tumours induced by the oncogenic adenoviruses.

The results indicate that adenovirus type 5, until now considered to be a non-oncogenic adenovirus, is capable of oncogenic transformation of cells *in vitro*. Attempts are being made to determine if the wild type virus or the *ts* mutants can induce tumour formation after inoculation of newborn hamsters.

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α-Bungarotoxin used as a Probe for Acetylcholine Receptors of Cultured Neurones

THE snake venom protein α-bungarotoxin binds with specificity and high affinity to nicotinic acetylcholine receptors of striated muscle[1-6], electric tissues[7-9], and mammalian brain[10]. For these reasons, this toxin, as well as similar

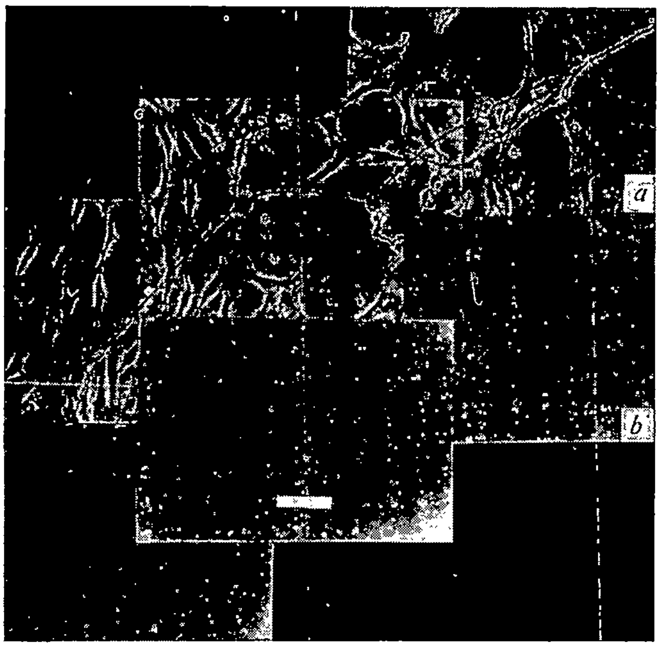

Fig. 1 Autoradiography of cultured cells from chick embryo sympathetic ganglia after incubation with ^{125}I-diiodo-α-bungarotoxin. Chains of paravertebral sympathetic ganglia from 11-day chick embryos (white Leghorn) were incubated in Ca, Mg-free Dulbecco's phosphate-buffered saline[18] containing 0.25% trypsin (Difco, 1 : 250) for 30 min at 37° C. The trypsin was removed and the chains were washed twice with complete culture medium (95% F-14 medium[19], 5% foetal bovine serum, and 10 ng (2 units) of nerve growth factor protein[17] per ml. of medium) and then dissociated by gentle trituration[15]. 1.0×10^5 cells were added in 2 ml. of complete media to collagen-coated 35 mm tissue culture dishes (Falcon). Maintenance of the cultures as well as their incubation with the labelled toxin was at 37° C in an atmosphere of 10% CO_2–90% air saturated with H_2O. The cells shown in the figures were maintained *in vitro* for 5 days. The growth medium was then replaced with 1 ml. of incubation medium (F-14 medium without choline and with 2 mg ml.$^{-1}$ of crystalline bovine serum albumin) containing 5×10^{-9} M ^{125}I-diiodo-α-bungarotoxin (2.2×10^5 Ci mol^{-1} toxin). After incubation for 1 h the dishes were washed at room temperature four times with 2 ml. portions of incubation medium and twice more with 2 ml. portions of F-14 (minus choline). Cells were fixed in 0.1 M sodium phosphate buffer (*p*H 7.4) containing 2.5% glutaraldehyde, and were subjected to autoradiography with Kodak NTB-2 emulsion for 8 days[20]. The same field is shown in *a* and *b*. Phase contrast optics were used to show cell structure (*a*), and bright field optics to show silver grains of the autoradiograph (*b*). The bar corresponds to 50 μm.

toxins from elapid snakes, has been used for labelling, purifying and assessing the physicochemical properties of cholinergic receptors.

Another possible use of α-bungarotoxin is to probe acetylcholine receptors of intact neurones. The toxin could be used to identify neurones possessing nicotinic receptors as well as to examine properties of the receptor such as concentration, distribution and pharmacology. Probing neuronal acetylcholine receptors in the intact nervous system is hindered, however, because individual neurones tend to be inaccessible to substances of high molecular weight and are difficult to view. One approach to this problem is to dissociate suitable neural tissue and to maintain it as a monolayer in cell culture. Neurones thus obtained and cultured may retain many differentiated properties[11,12], are readily accessible and easily viewed, and are in a relatively controlled environment. Neurones obtained from chick embryo sympathetic ganglia seem to have certain advantages as a model culture system. Sympathetic ganglion neurones *in vivo* are known to possess acetylcholine receptors of the nicotinic type (for reviews see refs. 13 and 14). Furthermore, chick sympathetic neurones can be obtained in reasonable numbers (approximately 2×10^5 neurones/chick embryo), are fairly easy to culture and are a relatively homogeneous population.

Here we report that ^{125}I-α-bungarotoxin binds to acetylcholine receptors of cultured chick embryo sympathetic neurones, and hence can be used to probe the properties of these receptors.

Chains of paravertebral sympathetic ganglia from 11-day chick embryos were dissociated with trypsin[15] and cultured in the presence of nerve growth factor[16,17] as described in the legend of Fig. 1. Cells with the appearance of neurones were quite distinctive after several days *in vitro*; their somas were round and highly refractile, and long, branched processes extended from them (Fig. 1*a*). Such cells did not survive *in vitro* in the absence of nerve growth factor. These cells also possessed electrically excitable membranes and responded to acetylcholine (A. Chalazonitis and L. A. Greene, in preparation). Hence, such cells have properties characteristic of differentiated sympathetic neurones. Other cell types, which probably include fibroblasts, capsule cells and glia were also present and formed a monolayer beneath the neurones within a week.

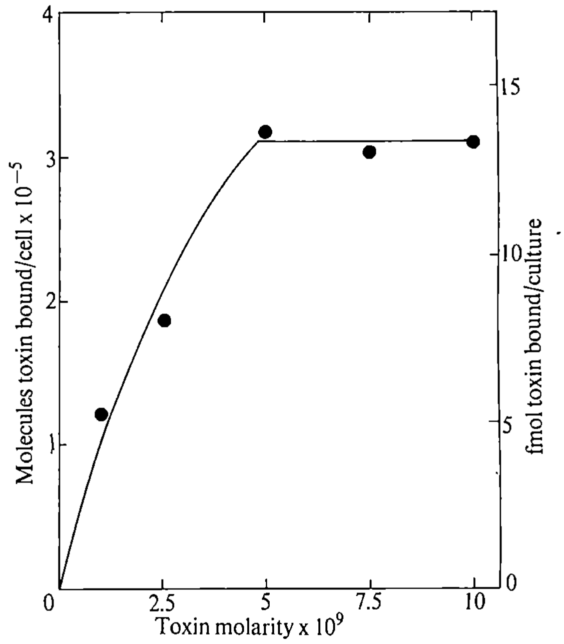

Fig. 2 The relation between α-bungarotoxin concentration and the amount of toxin bound to sympathetic neurons in cell culture. Cells dissociated from chick embryo sympathetic ganglia were seeded at 120,000 cells per dish and maintained *in vitro* for 5 days. The cells were then incubated with various concentrations of ^{125}I-diiodo-α-bungarotoxin (2.8×10^5 Ci mol^{-1} toxin) as described in the legend to Fig. 1. After incubation, cells were washed to remove unbound bungarotoxin, and bound radioactivity was determined as described previously[19]. Each value represents the average of triplicate determinations. Reproducibility in this and in other experiments was ±12%. The maximum amount of radioactivity bound was 5,600 c.p.m. In the absence of cells, approximately 100 c.p.m. bound to a collagen-coated Petri dish. This value was subtracted from values given in this and other figures. The number of neurones (termed "cells" in the ordinate) was determined as in subsequent experiments, by examining 4% of the surface area of three replicate cultures and counting neurones. The average dish contained 26,000±3,800 neurones.

The presence and specificity of a receptor for α-bungarotoxin on sympathetic neurones was examined by autoradiography. After various times *in vitro*, cultures were incubated in the presence of ^{125}I-diiodo-α-bungarotoxin. The preparation and characterization of the labelled toxin have been described previously[19]. The cultures were then washed, fixed and subjected to autoradiography[20]. A representative field of an autoradiograph photographed with phase contrast optics to show cell detail, and then with bright field optics to show silver grains, is presented in Figs. 1*a* and *b*, respectively. In this and other experiments (cells 2 to 22 days in culture), silver grains were localized over sympathetic neurones but not over other cell types. Both neuronal cell bodies and processes were labelled. All cells with neuronal morphology bound toxin but some seemed more heavily labelled than others. Furthermore, the distribution of silver grains over cell bodies and neurites was fairly uniform. No discrete regions with either relatively high or low concentrations of receptors were found, in contrast to the receptor clusters found with cultured chick embryo skeletal muscle[19,20].

The amount of α-bungarotoxin bound to cultured neurones was measured by determining the radioactivity retained by the cells after incubation with labelled toxin. Evidence that the radioactivity recovered was bound specifically to sympathetic neurones was obtained from the following observations. As previously pointed out, cultures without nerve growth factor lacked sympathetic neurones but not other cell types. Such cultures did not bind significant levels of labelled toxin. By contrast, cultures with nerve growth factor, and therefore containing neurones, bound the iodinated toxin.

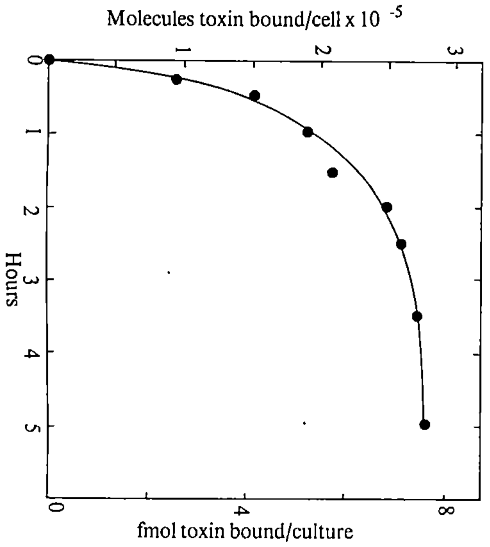

Fig. 3 Time dependence of the binding of labelled α-bungarotoxin to sympathetic neurones in cell culture. Cells dissociated from chick embryo sympathetic ganglia were seeded at 75,000 cells per dish, cultured for 5 days and incubated with 2.5×10^{-9} M ^{125}I-diiodo-α-bungarotoxin (2.6×10^{5} Ci mol^{-1} toxin) as described in the legend to Fig. 1. Cultures were then washed, and bound radioactivity was determined. Each value represents the average of duplicate determinations. The maximum amount of bound radioactivity was 3,200 c.p.m. The average Petri dish contained 16,500±3,000 neurones (termed "cells" in the ordinate).

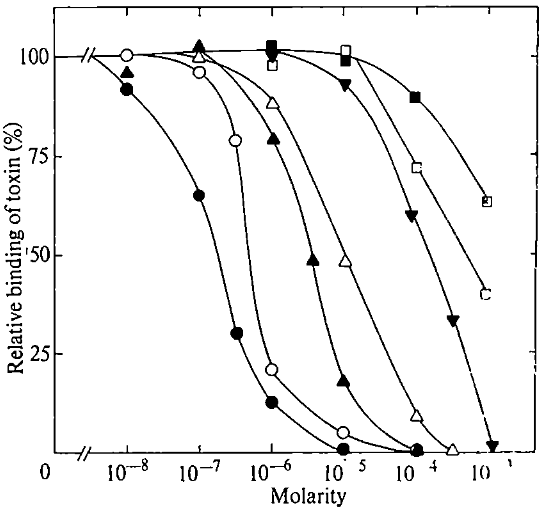

Fig. 4 The effect of ligands of acetylcholine receptors on the binding of ^{125}I-α-bungarotoxin to sympathetic neurones in cell culture. Cells dissociated from chick embryo sympathetic ganglia were seeded at 70,000 cells per dish and cultured for 4 to 5 days. Cultures were then incubated for 15 min in the presence of the desired final concentration of the appropriate cholinergic ligands. Then ^{125}I-diiodo-α-bungarotoxin (2.0×10^{5} Ci mol^{-1} toxin) was added in 2 μl. aliquots (final concentration in the medium$=1.25\times10^{-9}$ M) and the cultures were incubated for an additional 30 min in the presence of both labelled toxin and cholinergic ligand. Cultures were then washed, and bound radioactivity was determined. One hundred % on the ordinate corresponds to 4.7 fmol of toxin (1,500 c.p.m.) bound to cells in the absence of a cholinergic ligand. Each point represents the average of four or five determinations. ●, D-tubocurarine chloride; ○, nicotine dihydrochloride; ▲, 1,1-dimethyl-4-phenylpiperazinium iodide; △, acetylcholine chloride and 1×10^{-6} M eserine. sulphate; ▼, decamethonium iodide; □, hexamethonium chloride; ■, atropine sulphate.

Binding of the toxin was saturable. As shown in Fig. 2, after incubation for 1 h maximum binding was achieved at a toxin concentration of 5 nM or greater. From the maximum level of toxin bound per culture and from the number of neurones present, the average number of toxin receptors per cell was computed. Assuming that one molecule of toxin binds to one receptor and that the rate of dissociation of toxin from the receptor is relatively low, this number is about 3×10^{5} receptors per neurone. The average concentration of receptors on the neuronal membrane was also estimated. Assuming the sympathetic cell bodies to be smooth spheres 10 to 14 μm in diameter with cylindrical processes 0.6 μm in diameter and 500 to 1,000 μm in length (these values are averages measured for the cells in culture), the concentration of receptors on cultured sympathetic neurones is approximately 100–300 μm^{-2}. This concentration is similar to that estimated at extrasynaptic areas of striated muscle[5] and electric tissue[21] and at areas other than receptor clusters on cultured chick skeletal muscle[20], but is two to three orders of magnitude lower than the receptor concentration estimated at the corresponding synapses or clusters[3-5,20].

The rate at which the labelled toxin binds to cultured sympathetics is shown in Fig. 3. At a toxin concentration of 2 nM maximal binding was achieved after 3 h of incubation. A semilogarithmic plot of the rate data (assuming that binding is irreversible and that the toxin, is in great excess) yielded a rate constant, k_1, of 1×10^{5} M^{-1} s^{-1} for formation of the [toxin-receptor] complex. The rate of dissociation of the [toxin-receptor] complex remains to be determined.

Although comparative studies show some variation in receptor specificity among different organisms, selective cholinergic ligands are useful for characterizing and identifying acetylcholine receptors (see refs. 13 and 14 for reviews). Two classical types of acetylcholine receptor have been distinguished—nicotinic and muscarinic. In the mammal, for example, atropine selectively blocks muscarinic receptors, whereas D-tubocurarine selectively blocks nicotinic receptors. Furthermore, at least two varieties of nicotinic receptors have been found. Those of mammalian sympathetic neurones are selectively sensitive to hexamethonium, while those of striated muscle are selectively sensitive to decamethonium[22]. The pharmacological properties of avian sympathetic ganglion neurones have not been studied to our knowledge.

The specificity of the neuronal receptor for α-bungarotoxin was examined by determining the rate of binding of labelled toxin to cultured sympathetic neurones in the presence of various nicotinic and muscarinic ligands (Fig.

4). Experimental conditions were chosen so that binding of toxin (1.25 nM) was linearly proportional to the time of incubation. The rate of toxin binding was inhibited 50% by 0.25 μM D-tubocurarine; 0.6 μM nicotine; 3 μM 1,1-dimethyl-4-phenylpiperazinium; 10 μM acetylcholine in the presence of 1 μM eserine (an acetylcholinesterase inhibitor that had no effect on the binding at the concentration used); 200 μM decamethonium; and 500 μM hexamethonium. Atropine, at 1,000 μM, inhibited binding by only 30%. Also, binding of labelled toxin was completely inhibited by appropriate concentrations of most of the nicotinic ligands used in these studies as well as by a 100-fold excess (125 nM) of unlabelled toxin. Thus, binding of α-bungarotoxin to cultured sympathetic neurones was effectively inhibited by ligands of nicotinic acetylcholine receptors but not by a ligand of muscarinic receptors.

The relatively low potency of hexamethonium in inhibiting binding of toxin to cultured chick sympathetic neurones (50% inhibition at 500 μM) was somewhat unexpected in light of the potency of this agent in blocking nicotinic receptors on neurones of mammalian sympathetic ganglia[22]. Species variation between mammalian and avian ganglionic receptors may account for these differences.

The relatively low potency of decamethonium in inhibiting toxin binding (50% inhibition at 200 μM) provides further evidence for the specificity of acetylcholine receptors on cultured sympathetic neurones. In similar studies with α-bungarotoxin[19], acetylcholine receptors of cultured chick embryo skeletal muscle were at least 500 times more sensitive to decamethonium than were acetylcholine receptors of the cultured neurones reported here. Hence, the selective sensitivity to decamethonium observed with cultured cells resembles that reported for mammalian skeletal muscle and sympathetic ganglia *in vivo*[14,22].

These results suggest that the binding sites for α-bungarotoxin on cultured sympathetic ganglia neurones are nicotinic ganglionic acetylcholine receptors. *In vivo* this type of receptor on sympathetic neurones receives excitatory synaptic input from preganglionic neurones in the spinal cord. Alternate interpretations are possible, however. For example, the sites might be the cholinergic receptors suggested to be involved in release of catecholamines[23] or in propagation of nerve impulses[24].

In summary, α-bungarotoxin has been used to probe acetylcholine receptors of cultured sympathetic neurones. The distribution, concentration and pharmacological specificity of these receptors were studied. Systems such as the one described here should provide opportunities to identify specific types of neurones with acetylcholine receptors, to distinguish axons from dendrites, to examine the effect of one neurone on acetylcholine receptors of another, to study the regulation and turnover of neuronal acetylcholine receptors, and perhaps to study formation *in vitro* of certain types of synapses.

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Mutual Antagonism between Botulinum Toxin and β-Bungarotoxin

BOTH botulinum toxin[1,2] and β-bungarotoxin[3,4], isolated from the venom of *Bungarus multicinctus*, have been shown to block neuromuscular (NM) transmission by inhibition of the release mechanisms of the neurotransmitter. The inhibitory effect by either toxin is observed in the rat diaphragm only after a latent period, while the binding of both to the tissue seems to be almost complete during the latent period[3-5]. There seem to be two processes for the inhibitory effect, the binding of toxins to the tissue, and the subsequent changes leading to the irreversible inhibition of the release mechanism of neurotransmitter[4]. The time course of the paralysis is markedly accelerated by an increase of stimulus frequency and delayed by procedures or factors which depress the activity of release mechanism, such as deficiency of calcium, increase of magnesium or decreased rate of nerve stimulation[4-6]. By contrast with botulinum toxin (ref. 2 and our unpublished data), β-bungarotoxin shows an initial facilitatory effect[4], causing increases of the frequency of the spontaneous miniature end-plate potential and the quantal content of end-plate potential. Questions then arise as to whether or not they bind at a same site and act by a similar mechanism. We therefore studied the interaction between the two toxins in order to investigate the mechanism of the neurotransmitter release inhibition.

We used β-bungarotoxin, isolated by means of CM-'Sephadex' G-50 column chromatography[1], and crystalline botulinum toxin, type A, kindly supplied by Dr E. J. Schantz. The isolated phrenic nerve-diaphragm preparation of the rat was bathed in 50 ml Tyrode solution at 36° C and oxygenated with 95% O_2+5% CO_2. The nerve was stimulated with a single supramaximal rectangular pulse every 10 s and the time to cause complete NM blockade was measured. The relations between the time for complete NM block and the concentration of each toxin are illustrated in Fig. 1. The interaction between botulinum toxin and β-bungarotoxin was studied at concentrations of 0.1 and 3 μg ml^{-1} respectively. At the respective concentration both toxins blocked the transmission within 150 min when applied alone, but when both toxins were added at the same time, the paralysis was markedly delayed and NM blockade took place only after 250 min, corresponding to a reduction of activity of both toxins by about 90% (Table 1). The same antagonism was also observed when botulinum toxin was applied 30 min after the addition of β-bungarotoxin with or without simultaneous removal of the latter toxin (Table 1).

Table 1 Interactions Between Botulinum Toxin and β-Bungarotoxin

Treatment*		N	Time to paralysis† (min ± s.e.)	Relative‡ potency (%)
First	Second			
Botulinum toxin	—	12	147 ± 7	100
β-Bungarotoxin	—	3	126 ± 4	100
Botulinum + β-bungarotoxin	—	6	253 ± 27	13§, 5.2‖
β-Bungarotoxin, 30 min	Botulinum	5	217 ± 14	8.3
β-Bungarotoxin, 30 min then washed	Botulinum	4	237 ± 9	6.1
Botulinum, 30 min	β-Bungarotoxin	4	174 ± 13	46

* The rat isolated phrenic nerve-diaphragm preparations were first treated with botulinum toxin (0.1 μg ml^{-1}) or β-bungarotoxin (3 μg ml^{-1}), alone or in combination. The second treatment consisted of adding the other toxin 30 min later with or without removal of the first one.

† Time was taken from the addition of the first toxin.

‡ Relative potency of the first added toxin(s) was calculated by comparing with the concentration of uninteracted toxin which would take the same time to cause blockade (see Fig. 1).

§ Calculated against botulinum toxin.

‖ Calculated against β-bungarotoxin.

As the binding of β-bungarotoxin to the diaphragm was almost complete within 20 min[4], this result may indicate an antagonistic effect of botulinum toxin to the bound β-bungarotoxin rather than the formation of an inactive complex between the two toxins. The antagonism is unlikely to be the consequence of a competition or displacement at the binding site in the nerve endings, for either toxin bound to the receptive site would produce paralysis within 150 min by itself. Botulinum toxin evidently retards the inhibitory changes of transmitter release mechanism induced by bound β-bungarotoxin. Further, as the inhibitory effect of botulinum toxin was also decreased in the presence of β-bungarotoxin, it may follow that the binding of or the subsequent inhibitory changes by botulinum toxin is also counteracted by bound β-bungarotoxin. On the other hand, when botulinum toxin was first added and followed 30 min later by β-bungarotoxin, the time to complete blockade was also increased, but to a smaller extent (Table 1).

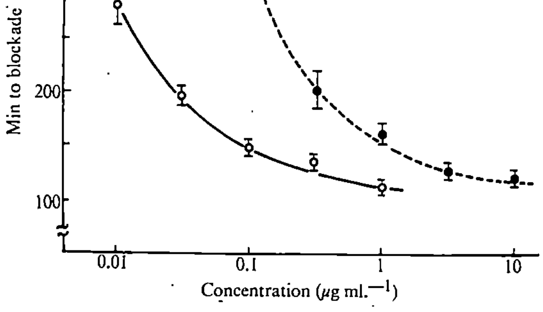

Fig. 1 Time to complete neuromuscular blockade of the rat isolated diaphragm preparation caused by various concentrations of either botulinum toxin (○—○) or β-bungarotoxin (● - - - ●). Vertical bars indicate the standard error of three to twelve experiments.

We suggest that botulinum toxin and β-bungarotoxin act at different sites although their presynaptic effects have many characteristics in common. Because the changes leading to inhibition of the release mechanism after binding with β-bungarotoxin can be retarded by addition of botulinum toxin, however, the binding sites for the two toxins must be closely related. In view of their opposite initial effects, the changes caused in the macromolecule which is involved in the release mechanism after binding of these toxins might be in opposite directions. The mutual antagonism could be thus obtained.

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Anomalous Pigments in the Eyes of the Red–Green Colour Blind

LORD RAYLEIGH noticed that his brother-in-law, Lord Balfour, had abnormal vision and he devised the Rayleigh match to examine it. In rather similar circumstances many years before, Clerk Maxwell used the Maxwell colour triangle to prove that his father-in-law lacked the fundamental red sensation.

Newton was the first to show that spectral red and green, suitably mixed, matched spectral yellow. Rayleigh used a modification of Maxwell's colour box to determine the proportions in which a fixed red and green must be mixed in order to match a fixed yellow. The instrument so used is called an anomaloscope. Rayleigh, whose vision was normal, found that all women agreed with him, but that about 5% of males disagreed, some requiring more red (protanomalous) some more green (deuteranomalous).

In his paper[1] he gives the names of his colleagues in the Cavendish Laboratory, Cambridge, who were abnormal, and it is interesting to note among the deuteranomalous, Sir J. J. Thomson, Nobel Laureate, who succeeded Rayleigh to the Cavendish chair.

Two kinds of explanations have been suggested for this common and much studied abnormality: (*a*) Perhaps the visual pigments which determine the eye's spectral sensitivity are abnormal. (*b*) Perhaps the pigments are normal, but there is abnormality in the way that their nerve messages are processed. Evidence favours (*a*).

The photoreceptors in the eye, like other photocells, can respond to lights of different wavelengths and are more sensitive to one colour than another. But there is only one dimension of output—the size (not also the quality) of signal—so any two lights can be matched to give identical outputs simply by adjusting their relative intensity.

It follows from this that if the Rayleigh match is so adjusted that the red cones are excited equally by the yellow field as by the (red+green) mixture, they will not be able to distinguish between them. And if at the same time the green cones are equally excited by each field, then they too will be unable to distinguish them and so the two fields will be altogether indistinguishable. (Blue cones are not excited at all by these lights.) This is the condition of the Rayleigh match. If the cone pigments cannot tell apart the two fields, nothing else in the visual system can..

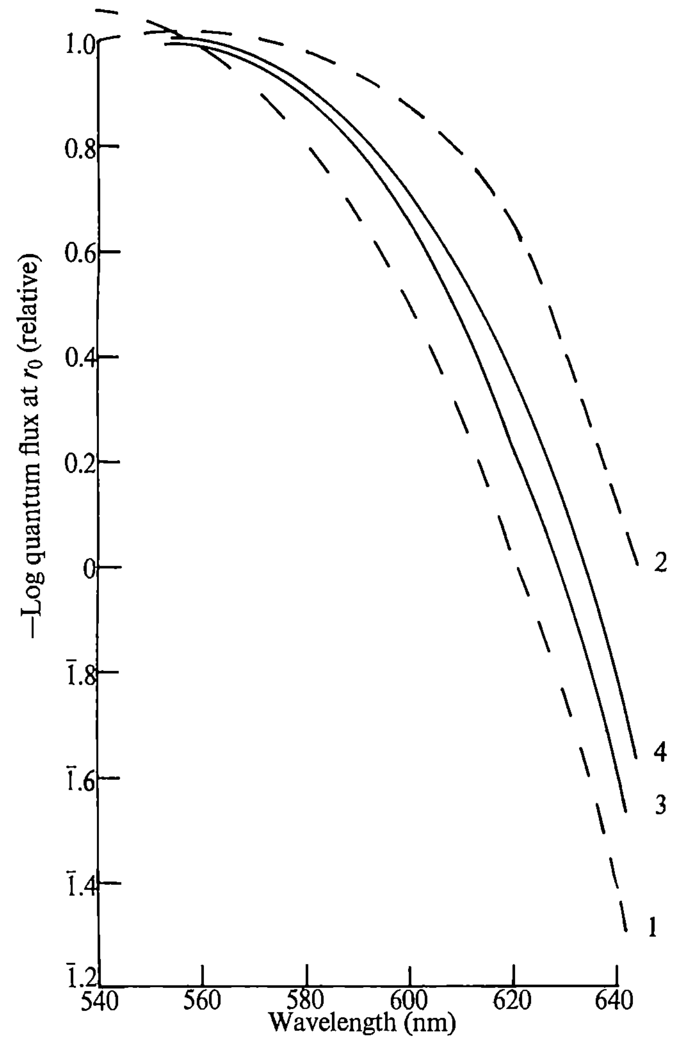

Fig. 1 Log spectral sensitivity curves of four cone pigments. 1, Chlorolabe; 2, Erythrolabe, the "green" and "red" pigments of normal eyes; 3, Protanolabe; 4, Deutanolabe, the pigments of anomalous eyes.

So if Rayleigh's abnormal subjects had normal cone pigments they could not distinguish between two fields that matched for normal pigments whatever the nerve processing. But this is precisely what they can do. Protanomalous insist that the mixture is too green, deuteranomalous that it is too red. They must each possess an abnormal pigment. Over the years, these pigments have proved elusive or their spectral sensitivities would have been discovered long ago. We have measured them at last; here we describe briefly how we succeeded. Full details will be published elsewhere[6].

For brevity we consider here only the protanomalous type; the deuteranomalous is exactly analogous. One pigment in the eye of the protanomalous is chlorolabe, the green pigment of the normal eye. It is found alone in the protanope, an extreme case who can match red with green. When he has matched these two lights exactly, naturally he cannot tell when they are exchanged. Thus when protanomalous (or normals) can tell that these lights have been exchanged, the information must come from the other kind of cone pigment. Consequently, in measuring the exchange threshold in the protanomalous, we measure abnormal cones only.

The exchange light was presented superimposed on a red or green background. Backgrounds always raise the threshold (Weber's law) and we found the relative energies of red and green lights that raised those exchange thresholds equally. Now Stiles[2–4] in his extensive studies of colour mechanisms in normal eyes, has shown that two background lights which for any mechanism raise the threshold equally are also equal in exciting that mechanism. We conclude that our red and green backgrounds were equal in exciting the abnormal pigment—the only one in operation at this exchange threshold.

With this information and Rayleigh matches (red+green) against not only yellow, but a set of monochromatic lights, it is possible to obtain the spectral sensitivity curve of the anomalous pigment. With our matching equipment (the Analytical Anomaloscope[5]) the answer is obtained directly.

The results are shown in Fig. 1 plotted as relative log spectral sensitivities in the red–green spectral range. Curve 1 is chlorolabe, present alone in the protanope, combined with 2 in the normal and with 3 in the protanomalous. Curve 2 is erythrolabe, present alone in the deuteranope, combined with 1 in the normal and with 4 in the deuteranomalous. Curve 3 is the anomalous pigment protanolabe, combined with 1 in the protanomalous. Curve 4 is the anomalous pigment deutanolabe combined with 2 in the deuteranomalous.

The combination 1 and 3 or 2 and 4 in the anomalous trichromat (instead of 1 and 2 in the normal) accounts for his abnormal colour matching, for the curves were obtained from these matchings. They also account for his poor colour discrimination. Clearly, the more nearly the two pigment curves coincide, the harder will it be for them to distinguish different wavelengths. Curve 4 lies close to 2, 3 even closer to 1, so these subjects have a very short "base" for wavelength discrimination. The similarity of their two curves has also made it hard to separate these two pigments by retinal densitometry and other methods. But by accurately neutralizing the activity of the principal cones, we have been able to measure the spectral sensitivity of the others.

Piantanida and Sperling (private communication) have found these same pigments by entirely independent research. Measuring increment thresholds against critically selected backgrounds they appear to have been able to isolate the anomalous pigments. We are surprised that this was possible, but because they have found the same pigments that we found, they must be right.

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Effect of Intravenous Prostaglandin $F_{2\alpha}$ on Small Intestinal Function in Man

ENDOGENOUS and exogenous prostaglandins (PGs) have been implicated in the pathogenesis of diarrhoea. $PGF_{2\alpha}$ may be secreted by some thyroid tumours which are accompanied by diarrhoea[1]; when given for the termination of pregnancy this PG produces diarrhoea as an undesirable effect[2]. Oral or intraluminal PGE_1 speeds up intestinal transit[3] and causes secretion of fluid and electrolytes into the intestine[4]. In animals, PGE_1 and $PGF_{2\alpha}$ inhibit intestinal sodium transport[5] and produce loss of fluid from intestinal loops[6]. Isolated human intestinal muscle responds to PGs with contractions or relaxations, depending on the site and the PG used[7–10]. In spite of these marked effects on both the secretory and motor functions of the gut, the action of intravenous prostaglandins on human small intestinal function has not been studied. We have measured the effect of intravenous infusions of $PGF_{2\alpha}$ on the net movement of water, electrolytes, transit rate and on pressure activity in the human jejunum and ileum.

Table 1 Mean Transit Time (min/30 cm of Intestine)

Study No.	Jejunum Control	Jejunum $PGF_{2\alpha}$	Ileum Control	Ileum $PGF_{2\alpha}$
1	*	8.8	13.1	12.1
2	10.7	7.4	8.0	8.9
3	4.9	7.0	10.0	8.5
4	6.3	7.0	12.6	12.9
5	7.5	8.8	13.1	11.3
6	6.1	8.8		
7	7.9	9.5		
8	*	*		
9	8.1	14.5		

* Curve unsatisfactory.

Intestinal movements of water and electrolytes were measured in the jejunum of nine and in the ileum of six healthy male volunteers by means of a continuous steady state perfusion system using a triple-lumen tube[11]. The position of the tube was confirmed radiologically immediately before the start of the perfusion. In the jejunal studies the infusion point was just distal to the duodeno-jejunal junction; in the ileal studies the infusion point was 65 cm proximal to the ileo-caecal sphincter. A 15 cm mixing segment and a 30 cm study segment were used; the infusion rate was 10 ml min^{-1} in all except three studies (jejunal studies 1, 3, 5). Two additional lumens perfused with water at the rate of 10 ml h^{-1} opened 5 cm apart in the study segment and were used to record intraluminal pressures. Transit through the study segment was measured with a dye injection technique[12] using 2 ml of phenol red (PSP) (6 mg ml^{-1}). The jejunum was perfused with a solution containing sodium and chloride 140 mmol l^{-1} and mannitol 20 mmol l^{-1}; the ileum with a solution containing sodium 135, potassium 5, chloride 110, bicarbonate 30 and mannitol 20 mmol l^{-1}. Both solutions contained polyethylene glycol (PEG) 1 g l^{-1} as an unabsorbable marker and were 300 mosM. Sodium chloride solution (0.9%) with or without $PGF_{2\alpha}$ was administered intravenously at a constant rate from a motor-driven syringe during the control and experimental periods respectively. Electrolytes and PSP were determined on an auto-analyser; PEG was estimated as described by Hyden[13]. Intraluminal pressure records were analysed in terms of the mean number of pressure peaks min^{-1} recorded during each of the 10 min collection periods. A pressure peak was defined as a rise above the baseline of intraluminal pressure of 2 mm Hg or more. From the start of the intestinal perfusion at least 60 min were allowed for the development of a steady state. After equilibration, four–six consecutive 10 min collections were made to provide control data. The intravenous infusion was then changed from saline to $PGF_{2\alpha}$ at the dose of 0.28–0.86 μg kg^{-1} min^{-1}, and after a further equilibration period of 30 min, four 10 min collections were made. The doses of PG were similar to those used in therapeutic abortion. The results expressed are the mean values of the last three consecutive 10 min collections and pressure measurements during each period of study. Transit time was measured during the control and PG periods.

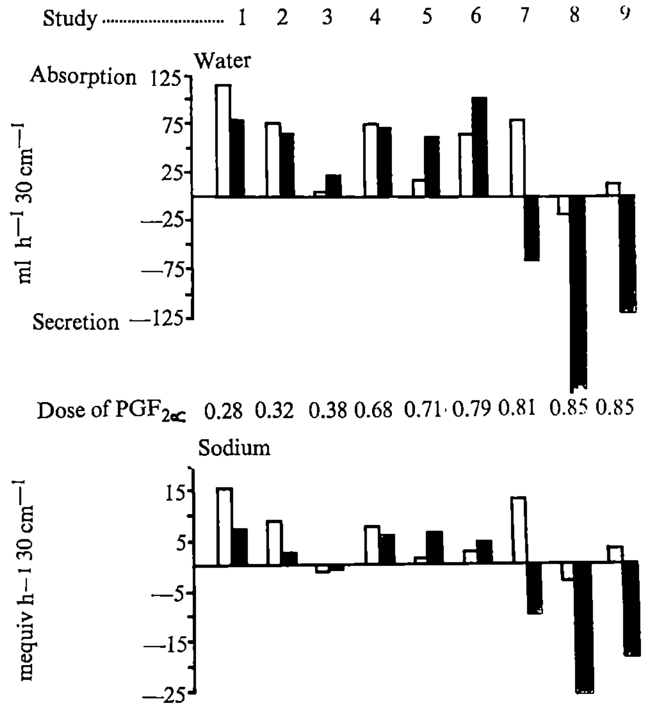

Fig. 1 Net water and sodium movements in the jejunum. Open blocks control, closed blocks $PGF_{2\alpha}$. Net absorption from the lumen above the line, net secretion into the lumen below the line. Mean values for the last 30 min in each study period are shown. Movements of chloride followed closely the movements of sodium.

In the jejunum net secretion of water, sodium and chloride into the lumen occurred in the three subjects receiving the highest doses of $PGF_{2\alpha}$ (0.81–0.85 μg kg^{-1} min^{-1}). Net secretion did not occur until 30–40 min after the start of $PGF_{2\alpha}$ infusion. No change was observed in the remaining six who received doses of 0.28–0.79 μg kg^{-1} min^{-1} (Fig. 1). Satisfactory measurements of transit time were available in seven subjects and although some slowing occurred during $PGF_{2\alpha}$ infusion in six of these, the change was not statistically significant (Table 1). The frequency of the segmental pressure waves in the study segment was markedly diminished in the four subjects receiving the highest doses of $PGF_{2\alpha}$. The inhibition occurred within 10 min of the start of $PGF_{2\alpha}$ infusion and persisted to the end of the study (Table 2).

In the ileum net secretion of water, sodium and chloride into the lumen occurred in the four subjects receiving the highest doses of $PGF_{2\alpha}$ (0.65–0.86 μg kg^{-1} min^{-1}), but no change was observed at the two lower doses (0.52 and 0.56 μg kg^{-1} min^{-1}). In this respect the ileum appeared more sensitive than the jejunum, but the onset of net secretion was similarly delayed. Bicarbonate loss into the lumen was observed in three of the four subjects who secreted (Fig. 2). Measurements of transit time (available in five subjects) showed no consistent change during the administration of $PGF_{2\alpha}$ (Table 1). Pressure activity in the ileum was profoundly diminished in the five subjects given the highest doses (Table 2). Inhibition was rapid in onset and was sustained during the infusion of $PGF_{2\alpha}$

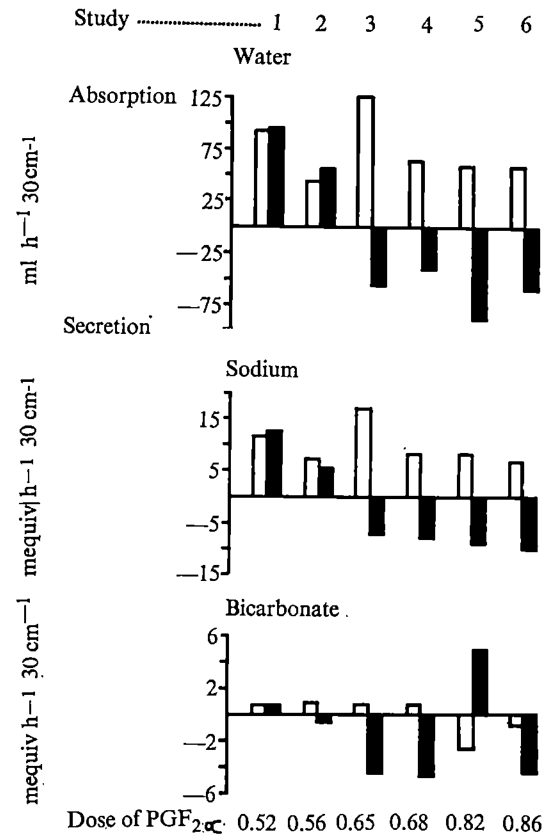

Fig. 2 Net water, sodium and bicarbonate movements in the ileum. Symbols as in Fig. 1. Movements of chloride followed closely the movements of sodium.

Table 2 Effect of $PGF_{2\alpha}$ on the Frequency of Segmental Pressure Waves

Study	Dose $PGF_{2\alpha}$ µg kg^{-1} min^{-1}	Secretion	Motility: pressure peaks min^{-1} Control Last 30 min	During $PGF_{2\alpha}$ First 30 min	During $PGF_{2\alpha}$ Last 30 min
			Jejunum		
1	0.28	−	2.3	2.5	2.5
2	0.32	−	3.9	3.3	3.1
3	0.38	−	2.3	2.9	3.1
4	0.68	−	1.6	1.3	1.3
5	0.71	−	1.6	1.7	0.4
6	0.79	−	2.5	0.8	1.5
7	0.81	+	3.4	0.4	0.5
8	0.85	+	5.0	2.0	0.3
9*	0.85	+	−	−	−
			Ileum		
1	0.52	−	3.2	2.8	2.5
2	0.56	−	2.1	0.6	0.8
3	0.65	+	4.6	2.0	2.7
4	0.68	+	3.6	1.6	2.0
5	0.82	+	2.9	1.1	0.7
6	0.86	+	5.0	1.1	1.3

* Pressure record was technically unsatisfactory in jejunal study No. 9.

(Fig. 3). In four subjects (studies No. 3 to 6) progressive contractions (mean amplitude in proximal tip 50 mm Hg, in distal tip 38 mm Hg) were recorded during the infusion of $PGF_{2\alpha}$, but their role in the propulsion of intestinal contents is uncertain.

During infusion of $PGF_{2\alpha}$ eight subjects experienced mild abdominal colic; this abdominal pain occurred in the presence of inhibition of small intestinal pressure activity. All but one of the subjects passed copious watery stools at the conclusion of the study. No changes were observed in pulse rates or blood pressures.

These results show clearly that intravenous $PGF_{2\alpha}$ profoundly affects the transport of water and electrolytes and the motor function of the small intestine, but it is uncertain which is more important in the pathogenesis of the diarrhoea. Both the jejunum and the ileum responded to $PGF_{2\alpha}$ by net secretion of fluid which was isotonic and approximated in composition to that normally found in these regions of the small intestine. Net movements of water and electrolytes into the intestine during infusion of $PGF_{2\alpha}$ were of a similar order to those observed in patients with cholera[14] and diarrhoea caused by *E. coli*[15]. Our data do not enable us to speculate on the mechanism whereby $PGF_{2\alpha}$ exerts its effect on fluid movement in the intestine. Net secretion did not, however, occur until 30–40 min after the start of $PGF_{2\alpha}$ infusion. This delay may be due to a rise in the concentration of circulating $PGF_{2\alpha}$ or active metabolites, but may also be explained by $PGF_{2\alpha}$ requiring a second messenger system to exert its effect.

Inhibition of small intestinal segmental pressure activity occurred sooner and at lower doses than the production of net fluid secretion into the lumen, suggesting a different mode of action by the $PGF_{2\alpha}$ on the muscle of the small intestine. This inhibition of motor activity may have been due to relaxation of the circular muscle. The circular muscle of the human ileum is inhibited *in vitro* by PGE_1 and E_2 (ref. 8). Our recording system did not allow us to measure changes in the activity of the longitudinal muscle layer, which *in vitro* is contracted by PGE_1, E_2 (ref. 8) and $F_{2\alpha}$ (ref. 9).

Propulsion of intestinal contents may be facilitated by inhibition of segmental pressures. Low segmental pressure activity has not been previously described in the small intestine during diarrhoea, although it occurs in the colon[16]. In spite of the diarrhoea, we were unable to record significant changes in transit time. On the other hand, the flow in the test segment increased by a mean of 1.46 ml min^{-1} in the jejunal, and by 1.26 ml min^{-1} in the ileal studies, in which secretion occurred: this represents an increase of some 15% above the rates of flow during the control period.

The presence of colic in spite of inhibition of small intestinal segmental pressures suggests that the colon may also be responding to infused prostaglandin: contractions of isolated human colonic muscle to $PGF_{2\alpha}$ have been reported[10]. Alternatively the colic and diarrhoea could result from the entry of a large volume of fluid into the caecum, with subsequent mass-movements and rapid propulsion of colonic contents.

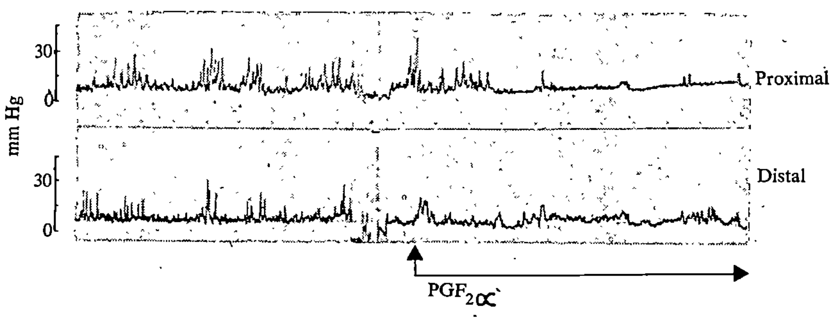

Fig. 3 Pressure activity in the ileum before and during $PGF_{2\alpha}$ infusion. Top trace proximal, bottom trace distal open-ended tube. Time marker, 1 min.

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Adult Parthenogenetic Chickens

Parthenogenetic chickens have been reported as embryos or chicks that lived to a maximum of eight days[1-3], except for one triploid White Leghorn chicken[4]. This chicken was from a virgin White Leghorn hen being tested as a control for a Dark Cornish-type stock which was under genetic selection for a high rate of parthenogenetic development. A cytogenetic study is in progress to determine the origin of parthenogenesis and to obtain parthenogenetic chickens.

In 1972, four parthenogenetic chicks were hatched from 8,532 eggs of the Dark Cornish-type stock[5] and raised to maturity. Three other chicks were hatched, but died during the first week. Average time to hatching was 24.4 days. These eggs were laid by virgin hens, sexed at six weeks of age and placed in separate pens. When the hens neared reproductive age they were placed in individual cages. No males were kept in the same room as the virgin hens. Special care had to be given to these chicks because they seemed to die as a result of gaseous inflated intestines, suggestive of enterotoxaemia. Therefore, as soon as the chicks started to pip the holes were enlarged and they were fed orally a mixture of 2,000 units of penicillin and 1 mg streptomycin each day for three days. The chicks were allowed to hatch normally the next day or were helped out of the eggs if they had difficulty. Some chicks had to be hand fed for the first two or three days. After this time, they were strong enough to feed themselves.

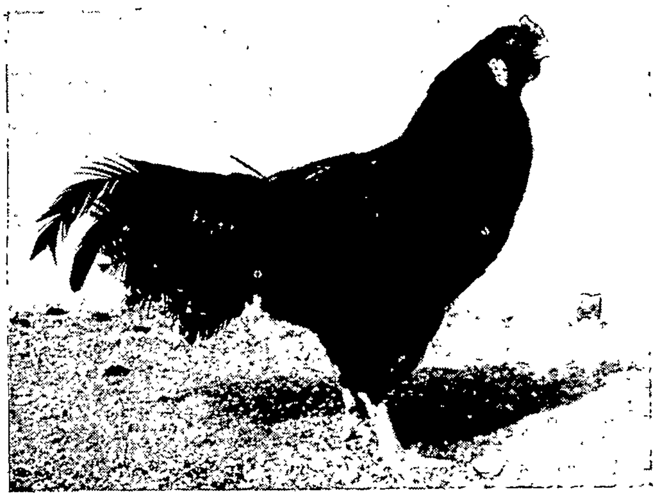

Fig. 1 Adult diploid parthenogenetic rooster.

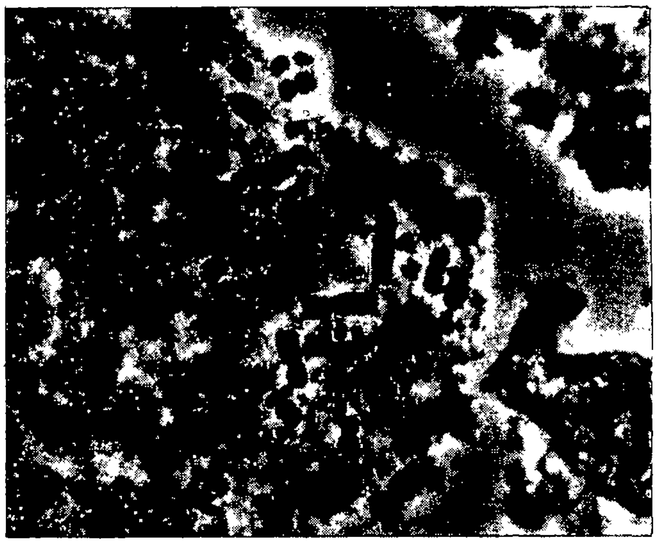

Fig. 2 Diploid cell from a parthenogenetic embryo. Note the ZZ chromosomes.

Four Dark Cornish-type chickens (Fig. 1) survived this treatment, and reached maturity. These birds are males and one mated with a White Leghorn female to produce fertile eggs. Chromosome counts from the feather pulp showed this male was diploid. Two of the other birds are also diploid. Fig. 2 shows a somatic cell from a colchicine-treated parthenogenetic embryo. The hen that laid this egg also laid an egg giving rise to one of the parthenogenetic roosters. The cell is diploid, and both ZZ chromosomes are present as they were in the parthenogenetic roosters that were raised. These are the first examples of adult diploid parthenogenetic chickens.

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Elimination Kinetics of α-Amino-iso-butyric Acid in Non-Pregnant and Pregnant Rabbits

CHANGES in the maternal metabolic and excretory functions occur during pregnancy; the plasma elimination rates of many compounds, such as folic acid[1,2], cortisol[3–5], nalidixic acid or gentamycin[6], have been found to differ in non-pregnant and pregnant subjects. Also, the plasma steady state concentration of most of the free amino acids is already lowered early in pregnancy. Here I report on the elimination rate of the model α-amino-iso-butyric acid (AIB) in pregnant and non-pregnant rabbits. I chose this amino acid because it is not metabolized[7,8]; it is transported and distributed throughout the body by the same mechanism as the naturally occurring free amino acids preferring the A-mediating system[9,10]; it is slowly and almost exclusively excreted by the kidneys[7,8,11]; and it is believed to accumulate in fast growing foetal tissue[12,13]. Twenty-one experiments were performed using nine rabbits. Ten experiments were carried out in the non-pregnant and eleven in the pregnant animal. The pregnant rabbits were investigated in the first and third weeks of gestation.

A single dose of tritiated AIB (^{3}H-AIB, specific activity 1.8 Ci mmol^{-1}) was administered intravenously. Blood samples were then drawn from the ear vein after 30, 70, 130, 180, 310 and 400 min. Two additional samples were collected after ~24 h and 1 week. The plasma ^{3}H-AIB concentration was measured in a Packard Tricarb Scintillation Counter.

Table 1 Plasma ^{3}H-AIB Concentrations at Different Times after Injection of a Single Dose (45 μCi) into Non-Pregnant and Pregnant Rabbits

Time after injection (min)	^{3}H-AIB concentration (c.p.s. ml^{-1}) (mean ± s.e.) Non-pregnant (n=8)	Pregnant (n=4)
30	57.2±2.9	68.7±5.8
70	42.2±3.0	51.1±9.1
130	33.9±2.1	37.6±3.8
180	28.9±1.8	30.9±2.6
310	23.2±1.6	26.6±2.4
400	21.3±1.4	23.0±2.2
24 h	9.8±1.2 (n=10)	10.3±0.7 (n=11)

Table 1 and Fig. 1 summarize my results. In the pregnant rabbits the plasma concentrations are generally higher and the elimination slightly slower than in the non-pregnant. There is, however, no statistically significant difference between the two groups of animals, and the results for the different stages of pregnancy are also similar.

The elimination curve of ^{3}H-AIB does not seem to be linear on the semilogarithmic scale and therefore first order kinetics are not applicable for the characterization of the disappearance of this substance from the plasma of non-pregnant and pregnant rabbits. It is, however, possible to show[14] that the experimentally obtained data closely fit a third order elimination model represented by

$$y = C_1 e^{-\alpha t} + C_2 e^{-\beta t} + C_3 e^{-\gamma t}$$

where the elimination constants are $\alpha = 0.52$, $\beta = 0.14$, $\gamma = 0.008$. Because I assume that the metabolic and excretory functions of the animals do not change during the periods of experiment, this indicates that the elimination of ^{3}H-AIB is determined simultaneously by the kinetic properties of three different compartments.

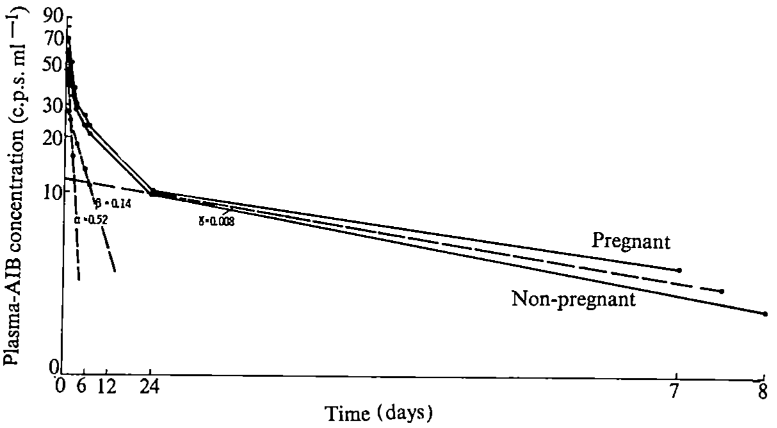

Fig. 1 Plasma elimination curve of ^{3}H-AIB in rabbits.

Henriques *et al.*[15], measuring the elimination rate of the naturally occurring free amino acid glycine in non-pregnant rabbits, came to similar conclusions. They were unable to find a single value for the plasma half-life of glycine and also suggested that at least three body compartments have an influence on the elimination kinetics of this particular amino acid.

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BOOK REVIEWS

Agriculture Under the Weather

Times of Feast, Times of Famine: a History of Climate since the Year 1000. By Emmanuel Le Roy Ladurie. Pp. xvi+428. Translated by Babara Bray. (George Allen and Unwin: London, January 1972.) £7.35.

It was in the late 1950s that the author of this book, then a young French economic historian engaged in research on the agricultural history of the sixteenth and seventeenth centuries in the Languedoc in the south of France, found it necessary to construct a history of the climate to explain his data. At the same time he found that the archives and documents he was using were extremely rich in weather data: for the peasants recording the poor harvests, famines, shortages and occasional years of abundance, had been intimately aware of the way in which their troubles were associated with the climatic stress of those times. Le Roy Ladurie's career over the years since has been a story of the enthusiasm and apparently boundless energy with which one historian, ignoring the alleged gulf between the two cultures of letters and science, applied himself to mastering all sources of information about past climate. The references in this book include the most relevant works in French, English, German, Russian and other languages besides, not only on local and regional history and archaeology, but also on vintages and tree growth and their climatic correlations, on pollen analysis, vegetation history and radiocarbon dating, the variations of the glaciers in Europe, Africa, America and the Arctic, and the blooming dates of the cherry in Japan, the variations of lakes in America and the Soviet Union as well as texts in hydrology, meteorology and oceanography. This praiseworthy effort has produced not only a realistic history of the environment, the background to human history which is still far too dimly perceived (if at all) by most human historians outside Scandinavia; it is also a book that is as charming, and appropriately illustrated, as it is informative.

Nor is this book only of academic importance in the unravelling and better understanding of the past. Scientists, too, who are confronted with the embarrassing demand for 50-year forecasts of the future trend of climate, need such reconstructions of the past record as Professor Ladurie has provided as the basic material of their studies. There can be no theoretical modelling of the long-term processes of climate without data. And for this purpose this book furnishes some tables of unusual length and completeness.

In detail, the book starts with the historical study of climate, this theme being expanded in chapter 2 by a consideration of the climatological evidence derived from tree growth (tree rings) and wine harvests. Two further chapters deal with the recent "amelioration" of climate over the half century ending about 1940, and with the problems of the "little ice age" between 1560 and about 1860, when glacier advances at least in the Alps were most spectacular and responsible for human distress around 1600, 1643–44, 1820, and 1850. A chapter follows on "working hypotheses" in which the available evidence from documents, instrumental records, and from wine harvest dates, tree-ring widths and so on, are synthesized and correlated with the observed climatic fluctuation over the past four centuries. A reconstruction of mediaeval climate from documents and from records of the Norse voyages to Greenland, radiocarbon dating of trees overwhelmed by glacier advances, oxygen isotope ratios in a deep core taken from the Greenland ice cap, phenological records and dates of the freezing of rivers receives a chapter to itself. All this leads to a final chapter on the physical causes and human consequences of climatic fluctuations.

From the start Professor Ladurie avoids the pitfalls inherent in the climatic interpretation of history followed by some of his predecessors. For example, wheat, wine and hay receipts for the estates of the Abbey of St Denis (Paris) were "steady as a gyroscope between 1284 and 1342". (Evidently the extreme wetness of the years 1313–1317 affected France much less than the rest of Europe.) Then the graphs of these receipts went "hurtling downward" after 1346–1348 because of the plague, the English and the brigands. Similar non-meteorological hazards ravaged the agriculture of north-eastern France during the same period. His analyses of the social and economic factors involved in addition to climatic conditions, in French wine growers choice of harvesting dates; besides his detailed descriptions of outstandingly cold epochs in France such as 1617–1650, with its critical glacial advances in the Alps in 1643–1644, are comprehensive and scholarly, and the book abounds with many other fascinating and illuminating examples of this sort. In justification for his detailed history of the "little ice age" he explains that earlier workers in this field have been scientists or geographers but not historians. Even writers "as reputable and competent as C. E. P. Brooks, coming from a country without glaciers . . . have fictionalized their "little ice age"; hence Professor Ladurie has worked in decades rather than centuries or longer.

A few minor criticisms can be made. On page 64, and occasionally on other pages, monthly climatological data make tedious reading and might have made more impact in tabular form. The author writes of "foggy wet summers" on page 74; probably a literal use of the source description. On pages 302 and 304 the word "meridian" should have been translated as "meridional", its English equivalent. Occasionally a diagram suffers in reproduction, Fig. 2 is one example, but in general the plates are beautifully reproduced and admirably supplement the text.

There is much evidence that the author has brought his work right up to date. For example, the dendrochronological work done at Tucson, Oxford and Caen is discussed (pages 40–42). He makes a vigorous plea (page 287) for more thorough collection and classification of vast numbers of scattered climatic references by computerization. Elated by the agreement he derives between wine harvest dates and glacier movements (page 79), he hopes that "young researchers in France, Hungary or Switzerland will work out the same sort of chronology on wine quantity for their own countries as Müller has provided for Germany".

The actual change in air temperature (about 1° C) between the little ice age and the recent "amelioration" in England, which seems small to the layman, makes about a month's difference to the length of the growing season. Both this and the cooling which has set in during the last two decades receive careful attention in Professor Ladurie's book, along with its pleasing production and printing.

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Excavation Under Water

Underwater Archeology: a Nascent Discipline. (Monuments and Museums, XIII.) Pp. 306. (Unesco: Paris, 1972.) 44 francs; $11 ; £3.30.

THIS book is in some respects aptly titled: underwater archaeology is a discipline which to a large extent is (as a dictionary defines "nascent") "in the act of being born, not yet mature". But it is misleading to apply a blanket description to so wide-ranging a subject, for, as the book clearly shows, some of the results so far obtained have been markedly less immature than others. For this reason it is particularly useful to find that the wide spectrum of results summarized here have been, in the main, chronicled by their various progenitors. The work of Professor Bass and his Pennsylvania University colleagues on ancient shipwrecks in the eastern Mediterranean bears comparison with the most exacting archaeological standards in or out of the water —as Bass himself stresses it should do. Likewise the well-known *Wasa* salvage, the excavation of wrecks from the drained polders of the Zuider Zee, the recovery of the Skuldelev viking ships from Roskilde Fjord, the Bremen Cog excavation, the work on ancient harbours and other submerged sites in the Mediterranean and elsewhere, and the fascinatingly precise sectioning of prehistoric remains in the Lake of Zurich, all weigh heavily on the credit side of the archaeological balance.

On the debit side, however, much work carried out over the past two decades on early wrecks on the eastern seaboard of the Americas seems to have been disturbingly inadequate and destructive, doubtless because privately sponsored treasure hunting appears to have been the primary motivation behind their discovery and excavation. Although structural remains of vessels dating back to the sixteenth century have been found there in some profusion, thus potentially covering an important and ill-documented period in the development of shipbuilding technology, Peterson's chapter gives no indication either in text or references of the existence of any published plans of such remains. To some extent the same criticism could be levelled at a good deal of what has been going on in our own waters, though the book, rather surprisingly, makes no mention of British work. It is worth reflecting that a close relationship exists between the quality of results achieved in a particular country and its government's degree of constructive involvement in this field: it is no coincidence that to date such involvement has been, both in the United States and in Britain, almost negligible.

The book includes useful chapters on techniques, not all of which, however, are wholly up to date. Williams's chapter on photogrammetric survey is practical and good, but Townsend's review of conservation procedures is less adequate, and—in common with the two other conservation chapters—does not mention important recent improvements in the treatment of waterlogged wood.

There is much in this book which is good, though a substantial amount is bad and more still is indifferent. This accurately mirrors the varying standards to be found within this new discipline as it endures its birth-pangs, and the book as a whole thus provides a useful survey of how the subject stands at present and where its strengths and weaknesses lie. As its editors themselves point out, it is a status report and not a textbook.

COLIN J. M. MARTIN

Applied Relativity

Gravitation and Cosmology: Principles and Applications of the General Theory of Relativity. By Steven Weinberg. Pp. xxviii+657. (John Wiley: New York and London, September 1972.) £8.

IN the last twenty years general relativity has developed dramatically in two opposite directions. On the one hand more powerful mathematical methods have clarified the general structure and significance of the theory, while on the other it has come into close contact with modern astrophysics. Put crudely, but not altogether inaccurately, we now have two subjects: topological relativity and engineering relativity. This book is unashamedly about engineering relativity. It is written by a man well known for his brilliant contributions to the theory of elementary particles, and for his occasional forays into relativistic astrophysics. In my opinion the book is quite superb as an introduction to its subject.

It begins with a brief, physically oriented, account of special and general relativity in sufficient mathematical detail for the later astrophysical applications. These applications begin with a treatment of stellar equilibrium and collapse. The level of this treatment is that of an introductory survey rather than a comprehensive account of the kind that can be found in Zeldovich and Novikov's book *Relativistic Astrophysics* or in various summer school lecture notes by Thorne. Nevertheless I would recommend it as an excellent introduction to the more detailed treatments. The book then reverts to a consideration of some mathematical developments used in general relativity, namely, concerning the action principle, the tetrad formalism and space-times which possess symmetries. It is not clear to me why this material is inserted into the middle of the astrophysical part of the book, rather than in the chapters on general relativity. It is true that the discussion of symmetries is of basic importance for the following section on cosmology, but it is also needed for the derivation of the Schwarzschild solution, where indeed a forward reference has to be made.

Finally we come to the cosmology section, which occupies the last two hundred or so pages of the book. The treatment here is up to date and has a physical orientation which I found very satisfying. Of particular value are the sections on intergalactic emission and absorption processes, the cosmic microwave radiation background, the thermal history of the early universe, and the formation of galaxies. In the final chapter there is a brief account of non-standard models of the universe which includes relativistic models with a cosmical constant, the steady state theory, and models with a varying constant of gravitation.

The book as a whole is clearly written, is pervaded by physical insight, and contains sufficient mathematical development to serve as an introduction to more detailed treatments and to the original literature. I recommend it to all physicists who want to gain a serious understanding of modern relativistic astrophysics, to students who wish to start work in this subject, and even to my colleagues.

I have left myself enough space to air one small quarrel which I have with the author. It concerns his attitude to the principle of equivalence and to Mach's principle. In the preface he states that the reason why gravitation obeys the equivalence principle is not to be found in classical physics but in the constraints imposed by the quantum theory of gravitation. In the body of the book he states that there is a sense in which the equivalence principle and Mach's principle are in direct opposition. I disagree with both these statements. Indeed I believe that Mach's principle precisely gives us the reason why gravitation does obey the equivalence principle, because it is the gravitational force of the distant accelerating masses which we interpret, with Mach, as an inertial force, and it is to be expected that the effects of this gravitational force on a physical system are fully equivalent to those of a (uniform) gravitational force of local origin. This conceptual question does not intrude elsewhere in the book, however, and I hope that it is widely read.

D. W. SCIAMA

Primate Anatomy

Evolutionary Biology of the Primates. By W. C. Osman Hill. Pp. x+233. (Academic: London and New York, December 1972.) £3.90.

THROUGH his monographic series, *Primates, Comparative Anatomy and Taxonomy*[1], W. C. Osman Hill has already performed a great service to primatologists by providing a basic reference source. However, there must be many who have hoped that he would also find time to write a short synthetic text for students, summarizing developments in the field of comparative anatomy since 1954 (the date of publication of his introductory text *Man's Ancestry*[2]). *Evolutionary Biology of the Primates* fills this gap with a succinct new review of Hill's encyclopaedic knowledge of primate anatomy. The book is based on a course of lectures designed for the NATO Advanced Study Institute on Comparative Biology of Primates, which was held in Turin in June 1972. Since the course was specifically designed for the training of primatology students, this is obviously the kind of book that one should consider for university teaching of primate anatomy and evolution, and it is inevitable that it will be compared with Le Gros Clark's *Antecedents of Man*[3], which has served as a basic text in this area for many years.

Hill's new book resembles that of Le Gros Clark in that it deals with primate anatomy in terms of separate organ systems. Indeed, a fair number of illustrations have been republished from *Antecedents of Man.* There is also the same heavy reliance on the structure of the skull and brain. However, Hill's approach differs in that, although the evolution of organ systems is considered in terms of different levels ("phases") of organization exhibited by living forms, the apparent linearity of this treatment is offset by separate discussion of the evolutionary radiation of the individual major groups of primates. A further significant advantage lies in the inclusion of a general section on the radiation of the placental mammals, placing primate evolution in its proper perspective — a perspective which is far too readily forgotten in many modern primatological studies. Thus, although *Evolutionary Biology of the Primates* and *Antecedents of Man* share many features of structure and content, there are enough differences to justify coexistence of the two texts as complementary aids to the study of primate anatomy. Hill's book is also more up-to-date in a number of respects.

It would have been better, however, to entitle this new book *"Comparative Anatomy of the Primates"*. There is no real discussion of evolutionary biology at any point, and there is very little reference to behaviour, physiology or biochemistry; in short, there is little mention of function. For example, the section on "Diet and Digestion" is almost exclusively concerned with the anatomy of the digestive tract, and not with function or the functional background to evolution. The only evolutionary model that is used in the text is that of the "phylogenetic scale", and Fig. 14 (page 19) shows "a series comprised of some living species arranged in an ascending evolutionary order". Although Hill points out that this "series" should not be taken literally, it does in fact form the primary basis for evolutionary inference throughout the book, for instance in the discussion of the evolution of the brain in terms of "phases" represented among living primates. The use of the phylogenetic scale concept is also prominent in the section on the placenta. Although it must be recognized that this scale concept has played a vital role in the development of primate evolutionary theory, its limitations should not pass unremarked[4,5]. In any event, many workers would question the implication (Fig. 14) that the orang-utan is more closely related to man than is the gorilla!

In view of the concentration on brain anatomy, it is unfortunate that there is no discussion of primate brain: body weight ratios, and no mention of the meticulous work carried out in this area by Stephan and Bauchot[6] over the last decade. The only reference to relative brain size is contained in a table (page 38) which misleadingly repeats individual measurements from 3 species, in one case derived from an apparently juvenile tree-shrew! In view of the evolutionary interest, it is also a pity that there is no mention of endocranial casts from fossil primates, since this provides the only direct evidence of brain evolution[7,8]. Finally, there is no mention of recent work on the primate retina, such as that of Wolin and Massopust[9]. These are all important omissions, and similar gaps occur in other parts of the book. For example, it is surprising that only 1½ pages are devoted to the key fossil primates of the Fayum, whilst less important fossils receive more detailed examination.

Thus, it must be recognized that this book has severe limitations as a basic text on primate evolution. Within the confines of these limitations, however, one is presented with a competent and well-illustrated text which is generally admirable in its clarity. The section on dental morphology in primates (pages 75–87), for example, is a model of clear explanation from first principles. Given the fact that this book also has a very reasonable price, it obviously has a broad appeal as a basic text in primate anatomy, and it should certainly be recommended to students as introductory reading, along with books covering other aspects of primate biology.

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Atmospheric Science

An Introduction to Air Chemistry. By S. S. Butcher and R. J. Charlson. Pp. xiii+241. (Academic: New York and London, November 1972.) $10.95.

THE contents of this book evolved from a set of class notes used by the authors in teaching. It is intended that the book be used by those with no previous experience in the field of atmospheric sciences as an introduction to the study of the chemistry and meteorology of the atmosphere. As its origins and title suggest, the book is set at the undergraduate level of study and as a textbook to accompany a lecture course it will be useful to both students and lecturers. The text is clear and presented in a way which will attract and retain students' interest. The authors usually appreciate that after an introduction many will need to go on to more detailed treatments and the necessary references and suggestions for further reading are included. In any textbook which attempts to bring together relevant aspects of meteorology, physics and chemistry the choice of material must inevitably be arbitrary and some readers will find the book contains less chemistry and rather more physics and meteorology than they might have expected. The title is, in fact, somewhat misleading and would be improved if the word "chemistry" in it were changed to "science".

The authors' claim that the book could be used as a reference text by workers in atmospheric chemistry is rather optimistic and at variance with the aims and achievement of what is essentially an introductory course. Similarly the use of the text by research workers new to the field of atmospheric science will be limited to the acquisition of a useful introduction and an indication of where more detailed treatments may be found.

The usefulness of some of the early chapters of the book as a text for teaching will depend to a large extent on the student's experience. The study of atmospheric science would probably fit best into the later stages of an undergraduate course and the summary of chemical principles in chapter 2 may well have been covered in much greater (and necessary) detail by that stage. The modern student would probably appreciate more detailed treatment of topics of contemporary interest such as heavy metals, halogens and the upper atmosphere, and the authors should consider their inclusion in future editions of this useful introductory text.

W. S. Clough

^{13}C Spectroscopy

Carbon-13 NMR Spectroscopy. By J. B. Stothers. Pp. xi+559. (Academic: New York and London, November 1972.) $24.

This book, written by an author who is well known for his ^{13}C n.m.r. work, is intended for organic chemists and others of graduate and research worker levels. Because the full potential of the ^{13}C n.m.r. techniques are just beginning to be exploited this book makes a timely and welcome appearance. Fourier transform n.m.r. spectrometers which are particularly useful for ^{13}C n.m.r. studies are now commercially available.

The book starts off with a short introductory chapter on the underlying theory and also makes a useful comparison with the well known proton method. This is followed by part 1, the first of four self-contained parts which consists of a chapter on the experimental measurement of ^{13}C parameters by both direct and indirect methods, and a discussion of their advantages and limitations is given. The statement that "pulse techniques will no doubt become popular" (page 19) seems to date the book somewhat. This no doubt reflects the surge forward in n.m.r. instrumentation during the time lapse which has occurred between writing and publication of the book.

The main part, part 2 (six chapters) consists of an extremely useful and comprehensive survey of ^{13}C n.m.r. shielding data covering the literature to mid-1970 in which all the shieldings are given relative to tetramethylsilane (with increasing positive numbers to lower fields). Data are presented for hydrocarbons and substituted hydrocarbons, organometallic compounds, carbonium ions, heterocyclic systems, carbonyl carbons and nitriles. In addition the current theoretical interpretations of the trends observed are discussed. This section helps to pinpoint those areas in which much further ^{31}C shielding data is required (for example for organometallic, sulphur and silicon compounds).

Part 3 (two chapters) considers the experimental determination together with the theoretical aspects of ^{13}C spin-spin coupling constants. A survey of mainly ^{13}C–^{1}H; ^{13}C–^{19}F and ^{13}C–^{13}C interactions is given which is not claimed to be comprehensive, because as the author rightly points out, the literature of even one bond ^{13}C couplings is enormous. More emphasis is placed on interactions over two or more bonds.

Part 4 (one chapter) consists almost entirely of examples using direct observation of ^{13}C spectra so as to illustrate the present usefulness and the potential scope of this technique. Structural elucidations together with stereochemical assignments dealing with geometrical isomerism and conformational analysis are first considered, followed by applications involving natural products illustrated with examples from the terpene, steroid, carbohydrate, nucleoside, nucleotide, amino-acid and peptide fields. Some applications involving polymers and petroleum are also given. Finally, the use of ^{13}C tracer techniques for the study of reaction-mechanisms and biosynthetic pathways and for solute-solvent interactions are discussed.

The book, which is well written and readable, contains in addition to the usual author and subject index, a useful compound index to shielding values and/or coupling constants given in the text. No research worker in the field can afford to be without this book on his laboratory shelf and I strongly recommend it to anyone interested in ^{13}C spectroscopy.

R. G. Rees

Internal Friction

Internal Friction of Structural Defects in Crystalline Solids. By R. De Batist. Pp. xii+477. (North-Holland: Amsterdam and London; Elsevier: New York, 1972.) Dfl. 120; $37.50.

A vast amount of information about the internal friction and associated properties of metals and other crystalline solids has been published during the past twenty years. This material has been reviewed in the series *Physical Acoustics*, edited by W. P. Mason, but that series contains many volumes and René De Batist correctly appreciated that there was a need for a single volume covering the techniques of internal friction measurements and their application to the study of lattice defects in crystalline solids.

The opening chapter surveys the basic properties of various structural defects; point defects and dislocations are considered in some detail while grain boundaries, domain boundaries and three-dimensional defects are briefly mentioned. Chapter 2 contains a formal description of anelasticity and a detailed discussion of relaxation phenomena, and concludes by describing a useful selection of the many different experimental techniques which have been used for measuring internal friction.

The remaining three chapters are concerned with internal friction caused by point defects, dislocations and two-dimensional defects respectively. In each case the author gives a comprehensive survey of the experimental data; this review of the experimental results is one of the most valuable features of the book. The theory of the relaxation of point defects, which is now fairly well established, is described clearly and in detail. By contrast, I was disappointed to find that recent modifications of Seeger's theory of the Bordoni peak are mentioned only briefly and qualitatively. The vibrating string model of dislocation resonance is discussed in detail, and there are useful sections on the various interaction effects between dislocations and point defects and on the application of internal friction techniques to the study of radiation damage in solids.

This book serves a valuable purpose in linking together in one volume the many aspects of internal friction which are often considered in isolation. The author has himself made valuable contributions in a range of different topics, and his experience has enabled him to select his material successfully and to discuss it in an authoritative manner. The book is well produced, with only a relatively small number of typographical errors, but it is a pity that its price is such that it may not reach the desks of all the research workers who might benefit from it.

D. H. Niblett

CORRESPONDENCE

Princeton in Trouble

Sir,—I think that your editorial "Princeton in Trouble" (*Nature*, **242**, 217 ; 1973) is highly damaging to the Institute of Advanced Study and the world of science in general.

First, I would point out the absurdity of the notion of social science. Science, as we all should know, consists foremost, of the application of the scientific method to the observation of natural phenomena. One of the essential requirements of scientific analysis is that it be possible to construct controlled experiments. In social studies of human beings, there is no possibility of constructing controlled experiments. Those who consider such a possibility should be strongly opposed. The second important requirement is that the experiments be repeatable. Since social situations are unique and cannot be repeated, social studies fail to meet the requirements of science on both counts. I would like to bring to your attention an excellent description of science in *Science is God*, by David F. Horrobin.

Next your suggestion "that there should be a better sense of moderation in this kind of scientific community" is immoral. If the standards of excellence and intellectual integrity are not maintained in such an academic community, then it is hard to conceive of any other place in the world where they would survive.

Your comment that "it is absurd for anybody at Princeton to suggest that a single appointment can entirely change the character of the faculty" is unfortunate and disputable. It is only single individuals that have been responsible for the course of human intellectual endeavour, to wit Socrates, Newton and Einstein.

Your comment that the dissident members' arguments are pernicious (the dictionary meaning of pernicious: extremely hurtful; wicked or mischievous) is slanderous. The faculty at Princeton does not possess such low qualities as you seem to do. It is editorials such as yours, and not their faculties that damage renowned institutions like the Institute of Advanced Study.

Yours faithfully,

S. Marathe

Rice University,
Houston, Texas,
77001

β-Carotene

Sir, — In response to McDonagh's answer (*Nature*, **241**, 151 ; 1973) to our earlier communication (*Nature*, **240**, 59 ; 1972), we offer the following comments.

In the interests of brevity, we did not belabour the distinction between topical and systemic administration of carotene. Since the scope of the discussion has now been broadened, it should be noted that Kesten made the logical progression from validating the efficacy of topical carotene, to oral administration of the compound, and that although exposure of the untreated patient's skin to a 'Mazda' projection bulb produced a wheal, the response could not be elicited after two weeks of oral therapy.

Admittedly, Kesten did not have the benefit of our present knowledge of the aetiology of erythropoietic protoporphyria (EPP), the *in vitro* photosensitizing action of protoporphyrin via singlet oxygen, and the quenching ability of β-carotene. But, despite convincing "test tube" evidence, there appears to be no experimental data relating to the *in vivo* state in humans[1]. McDonagh cited no evidence in support of his statement that the protective effect of β-carotene is enhanced when taken internally, and that the compound probably acts as an *in vivo* quencher of singlet oxygen as well as a light shield. On the contrary, the meagre information available suggests that oral β-carotene acts mainly (if not solely) as a screen to absorb harmful rays of the Sun, in a manner analogous to that of topically applied material. Thus, oral carotene does not provide photoprotection in EPP patients until serum concentrations reach massive levels[2] and the skin displays visible evidence of the presence of the carotenoid[1], which suggests to us that its mechanism of action is primarily that of a physical sunscreen. This conclusion is strengthened by the well-known observations that internally-administered carotene accumulates in the stratum corneum[3].

It is unfortunate that the *in vivo* protective action of β-carotene is not strongly enhanced by quenching ; the EPP patient whose situation is desperate enough to require massive carotene loading must pay the penalty of an unesthetic yellow colour in his skin. We have successfully treated EPP[4] and other types of photosensitivity[5] with topical application of dihydroxyacetone and lawsone (2-hydroxy-1,4-napthoquinone). This combination exhibits the property, shared by no other sunscreen, of binding chemically to the skin, and therefore does not require renewal during the patient's normal daily activities. The colour imparted to the skin is pleasing to the patients, and far more esthetic than the yellow hue of β-carotene.

Yours faithfully,

J. A. Johnson
R. M. Fusaro

University of Nebraska Medical Center,
Omaha, Nebraska 68105

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Ancient Egypt

Sir,—With regard to the recent paper by K. H. Hsu *et al.* (*Nature*, **242**, 240 ; 1973), I would like to point out that this theory has to some extent been anticipated by Herodotus (*circa* 485–425 BC) (Book II).

"They proved the truth of most of these assertions, and went on to tell me that the first man to rule Egypt was Min, in whose time the whole country, except the district around Thebes, was marsh, none of the land below Lake Moeris—seven days' voyage up river from the sea—then showing above the water. I have little doubt that they were right in this; for it is clear to any intelligent observer, even if he has no previous information on the subject, that the Egypt to which we sail nowadays is, as it were, the gift of the river and has come only recently into the possession of its inhabitants. The same is true of the country above the lake for the distance of a three days' voyage; the priests said nothing to me about it, but it is precisely the same type of country.

"Now, it is my belief that Egypt itself was originally some such arm of the sea—there were two gulfs, that is, one running from the Mediterranean southwards towards Ethiopia, and the other northwards from the Indian Ocean towards Syria, and the two almost met at their extreme ends, leaving only a small stretch of country between them. Suppose, now, that the Nile should change its course and flow into this gulf—the Red Sea—what is to prevent it from being silted up by the stream within, say, twenty thousand years? Personally, I think even ten thousand would be enough. That being so, surely in the vast stretch of time which has passed before I was born, a much bigger gulf than this could have been turned into dry land by the silt and brought down by the Nile—for the Nile is a great river and does, in fact, work great changes. So I not only believe the people who gave me this account of Egypt, but my own conclusions strongly support what they said. I have observed for myself that Egypt at the Nile Delta projects into the sea beyond the coast on either side; I have seen shells on the hills and noticed how salt exudes from the soil to such an extent that it affects even the pyramids; I have noticed, too, that the only hill where there is sand is the hill above Memphis, and—a further point—that the soil of Egypt does not resemble that of the neighbouring country of Arabia, or of Libya, or even of Syria (the Mediterranean coast of Arabia is inhabited by Syrians), but is black and friable as one would expect of an alluvial soil formed of the silt brought down by the river from Ethiopia. The soil of Libya is, as we know, reddish and sandy, while in Arabia and Syria it has a larger proportion of stone and clay. I had from the priests another striking piece of evidence about the origin of the country: namely, that in the reign of Moeris the whole area below Memphis used to be flooded when the river rose only twelve feet—and when I got the information Moeris had been dead for less than nine hundred years."

Yours faithfully,

D. B. JAMES

The Doctors' House,
Claremont Road,
Marlow,
Buckinghamshire

Announcements

Miscellaneous

The following have been elected Foreign Members of the Royal Society.

Professor John Bardeen, **University of Illinois**; Professor Manfred Eigen, **Max-Planck Institute of Physical Chemistry, Göttingen**; Professor Francois Jacob, **Pasteur Institute, Paris**; Professor Ulf Svante Von Euler, **Karolinska Institute, Stockholm.**

The following have been elected Foreign Associates of the National Academy of Sciences, Washington.

Sune Bergstrom, **Karolinska Institutet,** Stockholm; Albert Eschenmoser, **Technishe Hochschule,** Zurich; Vladimir A. Engelhardt, **Institute of Molecular Biology,** Moscow; Dennis Gabor, **Imperial College,** London; David V. Glass, **London School of Economics,** London; Jean Goguel, **Ecole des Mines,** Paris; Motto Kimura, **National Institute of Genetics,** Japan; George Klein, **Karolinska Institutet,** Stockholm; Ben Roy Mottelson; **Institute for Theoretical Atomic Physics,** Copenhagen; V. Ramalingaswami, **Institute of Medical Sciences,** New Delhi; Andrei D. Sakharov, **Lebedev Institute of Physics,** Moscow; I. S. Shklovsky, **Institute of Space Research,** Moscow.

University News

Dr Fredos Papangelou, **University of Göttingen,** has been appointed to the **Chair of Mathematical Statistics** at the **University of Manchester.**

Dr Eric Craig Easson, **University of Glasgow,** has been appointed to the **Chair of Radiotherapy** at the **University of Manchester.**

Dr Peter Joseph Mittler, **Pembroke College, Cambridge,** has been appointed **Professor of the Education of the Mentally Handicapped,** at the **University of Manchester.**

International Meetings

May 25–26, **Stress in Farm Animals.** (Honorary Secretary, Veterinary Field Station, Easterbush, Roslin, Midlothian).

May 30–June 1, **Ducted Propellers.** (Secretary, Royal Institution of Naval Architects, 10 Upper Belgrave Street, London SW1X 8BQ).

June 7–8, **Symposium on The Biology of Hystricomorph Rodents.** (The Assistant Director of Science (UM), The Zoological Society of London, Regent's Park, London NW1 4RY).

June 13–15, **International Conference on Hydrostatic Extrusion.** (Conference Organizer, Birniehill Institute, National Engineering Laboratory, East Kilbride, Glasgow G75 0QU.)

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NATURE Volume 243
MAY 25 1973

Who Should be Tracked Hovercraft's Heir?

THE Select Committee on Science and Technology has yet to unravel the full mystery associated with the decision taken earlier this year to close down Tracked Hovercraft Limited. Ever since the decision to abandon work at the Earith base of the company was announced, most unfortunately at a time when the select committee had just decided to investigate the future of the project, Mr Airey Neave and his committee have apparently left no stone unturned in their quest for the reasons behind the decision. But, as last week's hearings showed (see page 180) the committee still feels that there is more to be learnt and although its attempts to obtain the minutes of meetings held in the summer and autumn of 1972 might meet with stiff resistance Mr Neave and colleagues will certainly not rest until they are satisfied they know all the facts.

One uncertain aspect of the decision to stop further financial support for the hovertrain is who carried out an appraisal of the company before the final decision was taken. Last week both the chief scientist at the DTI, Dr Ieuan Maddock, and the Director General of Research at the Department of the Environment, Mr D. J. Lyons, did not, in the eyes of the select committee, provide a satisfactory answer to this question. Mr Neave's whimsical aside, on whether it will ever be known who evaluated the future prospects of the company summed up the present situation admirably. But the important aspects of the present inquiry are not solely concentrated around this issue. The deed is done and the decision taken in February to preserve the linear motor work of Tracked Hovercraft Limited through a contract with Hawker Siddeley Dynamics, the precise details of which have still to be determined, needs careful consideration.

According to the government, there is no future for an inter-city transport system based on the tracked hovercraft principle in Britain before 1985, and only limited possibilities between then and the end of the century. It is hard to argue with this although there are some who would say that British Rail should not be basing its future policy solely on the steel wheels on steel rails principle. But it is this conclusion which spelled the end for THL.

But unfortunately the decision did not turn out to be as simple as that and two factors have clouded the issue. First, the decision of the DTI to preserve the work carried out by the company of linear motors and, second, the fact that at the time of the decision to stop supporting THL, the company, in conjunction with Hawker Siddeley, were bidding to build a transit system for the Ontario Government in Toronto.

In the past few weeks both these explanations have been put forward as the rationale for the Department of Trade and Industry's decision to grant £500,000 to Hawker Siddeley to pursue work on linear motors and, unfortunately, both explanations leave something to be desired. Britain, primarily through the efforts of Professor E. R. Laithwaite at Imperial College, is in the forefront of research in linear motors, and it is proper that development work on linear motors should continue. But is Hawker Siddeley the right company for this? Hawker Siddeley has a redoubtable reputation in the fields of transport and the management of large projects, but palpably no experience of linear motors design and construction. If the object was now to go ahead and build a transport system based on linear motors then there would be little argument that the correct company had been chosen. But the object of granting money to Hawker Siddeley, according to the DTI, was to preserve the research work undertaken by THL on linear motors. A strange decision—if this was the only motive behind it—a company with experience in linear motors would clearly have been a more suitable choice. But it is now abundantly clear that preserving the linear motor research of THL was not the chief reason why Hawker Siddeley was chosen to be the beneficiary of the DTI's generosity. And it was the need for the combined Hawker Siddeley and Tracked Hovercraft Limited bid for the Ontario contract to still have credence after THL ceased operations that led to the DTI's actions.

The DTI is now in an awkward situation. Hawker Siddeley, as luck has it, unfortunately failed to win the Ontario contract—not, as Dr Maddock said last week, because of the demise of THL but because it clearly overbid on the civil engineering side. But the company can still look forward to £0.5 million of government money.

It is now time for the DTI to show that it is really serious about preserving the THL work on linear motors by looking to other companies which have had some previous experience with these motors. It is indeed fortunate that the arrangement with Hawker Siddeley still leaves the DTI open to place contracts elsewhere, and one of the pleasing aspects of last week's hearing was the announcement by Dr Maddock that his department is also talking to GEC, a company with some experience of linear motor work. This will be a more sensible way to proceed now that it is clear why Hawker Siddeley was chosen in the first place. What will indeed be of interest will be the terms of the contract between the Department of Trade and Industry and Hawker Siddeley when they are finally settled.

100 Years Ago

AT the special request of Rear-Admiral Sands, the U.S Congress, at its last session, allowed an appropriation for the purpose of completing and publishing the catalogue of southern stars, observed by Gilliss in 1850–52, and the work is now being put in the hands of computers for publication as soon as possible.

From *Nature*, 8, 94, *May* 29, 1873

OLD WORLD

Inquiry Continues into Hovertrain's Demise

If nothing else, last week's hearing by the Select Committee on Science and Technology as part of its inquiry into the decision to abandon the hovertrain project established that Dr Ieuan Maddock, chief scientist at the Department of Trade and Industry is an avid reader of Professor Eric Laithwaite's scientific papers. But Dr Maddock is also interested in what Professor Laithwaite says and he took exception to the statements made to the select committee three weeks ago by Professor Laithwaite about a conversation between the two men at the Royal Institution in February (see *Nature*, **242**, 490 ; 1973).

Professor Laithwaite claimed that Dr Maddock had "clenched his fist and said 'I wish that I had known all this sooner'" when he was shown a demonstration of a new linear motor developed by Professor Laithwaite that produced both lift and propulsion. Dr Maddock, who made it clear in no uncertain terms that he did not like being quoted second hand in public, said that the situation was nothing like Professor Laithwaite claimed. Perhaps, said Dr Maddock, he had clenched his fist, but it was not over being surprised with the demonstration, but probably because Professor Laithwaite had mentioned that he had applied to the Science Research Council for a grant to pursue the work. Dr Maddock being a member of the SRC was surprised that he was not aware of the application.

Dr Maddock and Mr Lyons, Director General of Research at the Department of the Environment, who also gave evidence to the committee, displayed a remarkable degree of coolness towards the proved linear motor developed by Professor Laithwaite and his team at Imperial College in the past few years. It is far from clear, said Dr Maddock, whether in fact building a transport system based on one of these new motors would be more economical than building a system based on the older type of linear motor. And, said Dr Maddock, there were great difficulties associated with the new motor—in particular the repulsion which provides the lift is known to change into an attraction at high speeds. Such problems had to be solved before the new motor could be considered as the basis for a transport system.

Apart from bringing out this particular disagreement between Dr Maddock and Professor Laithwaite, the select committee also spent some time delving into the reasons why the work of Tracked Hovercraft Limited was halted. It emerged that although the decision was announced in February 1973, a meeting at which Tracked Hovercraft Limited, the NRDC (through which the DTI sponsored THL) and Hawker Siddeley were represented was held in August 1972 with follow up meetings held in October and November. It is the minutes of these meetings which the select committee is now trying to obtain in order to satisfy themselves of the reasons why support was cut off from THL and also, especially, to discover who, or what group of people, carried out an analysis of the future of THL.

Why was Hawker Siddeley the only industrial company represented at the August meeting, the select committee asked. After a suitable period for thinking and note passing the answer was that the views of industry on the prospects for the work of THL had to be represented and Hawker Siddeley was chosen to represent those views. Before Hawker Siddeley was awarded £500,000 last February to continue the THL work on linear motors, other companies were consulted and were represented at the later meetings, according to Dr Maddock. But the precise way in which Hawker Siddeley is going to spend the

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Nature 1972 Index

The combined index to *Nature*, *Nature New Biology* and *Nature Physical Science* for 1972 is now available. A subscription form is to be found on page iv.

BRITISH ANTARCTIC SURVEY

Icy Problems

The disappearance over the side of the research vessel Bransfield of biological samples from the Antarctic has put back parts of the British Antarctic Survey's work by a year ; replacing the lost samples could cost as much as £20,000, according to Sir Vivian Fuchs, the survey's director.

No charges have yet been made in connexion with the loss, and the two members of the ship's crew who were helping police with their inquiries at Shirley police station in Southampton last week have been released, pending the result of forensic studies.

The loss of the samples was discovered after the Bransfield, returning from the Antarctic, had left Montevideo on its way home. A survey officer flew out to meet the ship in Las Palmas, but the police were called in when he was unable to clear up the problem.

Apparently the samples were thrown overboard following a dispute over whether beer should be stored in the samples fridge in the Bransfield's scientific laboratory. The fridge contained assorted biological samples which were maintained at −40° C, a temperature considerably lower than that of the domestic refrigerators on board. Andrew Clarke and Michael Richardson, two members of the survey team, objected to the constant opening and closing of the fridge door to get beer out, as, in the hot temperatures of the tropics their samples were likely to suffer. The crew were finally told to take their beer out of the fridge, and the laboratory was locked. But next day it was found that the laboratory had been broken into and the samples had gone.

The samples included assorted crustacea and algae, including some examples of *Boeckella sylvestri*, fresh moss, mineral ash, marine benthos and extracted lipids, as well as whole and dissected birds and fish, and blood and tissue samples taken from expedition members at the Signy Island base which were to be used in the physiological studies of the effects on man of living at extremely low temperatures for extended periods. Samples of *Notothenia neglecta*, the "Antarctic cod", and *Chaenocephalus aceratus*, one of the Antarctic "ice fish" which live in the very cold waters close to the ice shelf and which are "bloodless", having no haemoglobin, the oxygen being transported round the body in their blood plasma, were also lost.

The lost samples represent more than a year's work for the two biologists, and affect the work of four other scientists still at Signy Island. Clarke and Richardson, both of whom had been in the Antarctic for two and a half years, were there to write reports in the British Antarctic Survey's scientific series, and would probably have used the results of their work to apply for PhD degrees at a British university once the full studies on the specimens had been completed at Monks Wood research station. As it is, much of the work will have to be repeated.

money from the DTI has yet to be determined—according to Dr Maddock the money was awarded on the basis of agreement and the details are being worked out at present.

But was Hawker Siddeley awarded this money solely to develop research on linear motors? It seems not, for it became clear at the select committee meeting that the DTI was concerned at the beginning of the year that the demise of THL could jeopardise the combined THL and Hawker Siddeley bid to build a transport system in Toronto, Canada. So to a certain extent the money was awarded to maintain the credibility of the bid. Since then, of course the Toronto contract has been awarded to Krauss Maffeii a German company.

But the contract with Hawker Siddeley is not exclusive and the DTI is at present discussing with GEC the possibilities of it doing some work on linear motors.

MARITIME SATELLITE

GTS May Not Do

EUROPE is unlikely to be taking a share in Britain's Geostationary Technology Satellite. At a meeting of the European Space Research Organization's joint programmes and policy committee last week, a comparison between GTS and an adaption of ESRO's own Orbital Test Satellite as possible maritime satellites was presented which heavily favoured the adoption of the ESRO satellite rather than GTS.

No decision has yet been taken in Europe to develop a maritime satellite to improve ship to shore radio contact, but ESRO has been undertaking studies for some time, and a performance specification for a maritime satellite has been drawn up, which has been largely approved by the Intergovernmental Maritime Consultative Organization (IMCO).

Britain offered GTS as a possible base for a test maritime satellite at last December's European Space Conference in Brussels at which the decision to attempt to rationalize Europe's space programme was taken. But ESRO's assessment of it argues that its facilities would not be adequate as a pre-operational satellite when compared to the facilities that ESRO's adapted Orbital Test Satellite could offer. GTS, being a completely new project, would also cost more, ESRO says.

GTS at present is still in the project definition stage. Conceived in mid-1971, its aim was to demonstrate high quality communications links and operative experience of various communications services, as well as to allow investigation of the propagation of radio frequency waves and of new British spacecraft systems.

The offer of it as a pre-operational maritime satellite falls down chiefly because it is designed as an experimental technology satellite which will provide some operational experience, rather than as an operational satellite which also provides some experimental facilities, which is the concept behind the ESRO satellite.

It is not entirely surprising that ESRO's proposal meets the performance specification rather more closely than GTS. When the GTS proposal was submitted at the end of March the specification had not been drawn up, and ESRO's secretariat drafted the specification at the same time as the specification for ESRO's own proposal was worked out.

But given the performance specification that has emerged from ESRO's consultation with interested parties and with IMCO, GTS plainly fails to meet it.

The performance specification calls for a satellite with 17.5° "global coverage" antenna beams to service an area stretching from Panama to the Persian Gulf in order to cover the principal tanker routes and 50 per cent of the world's chief shipping movements. These antenna should provide satellite to ship and satellite to shore links. GTS, however, can only provide a 7° antenna beam designed to cover the North Atlantic in the satellite to ship band, and a 1° beam in the satellite to shore band—a beam that could only cover a small part of Europe. GTS also only provides eight channels, whereas the specification calls for sixteen, although GTS does provide experimental facilities to use time division multiple access (TDMA), which IMCO has urged should be studied, whereas ESRO's proposals can provide only frequency division multiple access (FDMA).

Nonetheless ESRO's proposal meets the MARSAT specification and GTS does not.

But whether the British proposal will in fact find favour when the choice between the two systems is raised at the ESRO council meeting planned for June 1, will depend on several factors. Partly it will depend on whether the council wants an experimental or an operational capability (and the latter seems more likely) and partly on the results of the inevitable bargaining that will take place on other European space ventures such as Spacelab, L3S, (the new launcher proposed by France) and the need to spread Europe's money as far as possible. One benefit from Europe's point of view in the British proposal is that Britain will shoulder 75% of the cost. If ESRO's version of a maritime satellite is built, Britain would not have to provide more than the Gross National Product share (23%) that she normally puts into ESRO programmes.

SOVIET SCIENCE

Future Difficulties?

from our Soviet Correspondent

THE forthcoming meeting of the joint Soviet–United States team to discuss the Soyuz–Apollo project, which is scheduled for 1975, will take place under what are undoubtedly unfavourable conditions as far as the Soviet side is concerned. Although no official statements have appeared in the Soviet press two major setbacks occurred to the Soviet space programme last month.

Over the years, the Soviet space programme has had more than its fair share of failures. Some, such as the abortive launch on November 26, 1965, of a Venus probe, were "disguised" under the cover-all of the Kosmos programme. Failures on the launch-pad apparently go unreported, although reconnaisance satellites from the United States have observed four "disappearances" from the launch-pad of large rockets which have never gone into orbit. The most recent of these was at the end of November 1972. Even the failure last month of a major lunar probe, carrying what should have been Lunokhod-3, has passed without official comment. Only when loss of life is involved does Baikonur admit failure.

The failure of the Salyut-2 station, however, could not pass unremarked. A successor to the Salyut-1 of 1971, its launch was reported in *Pravda* on April 4 accompanied by an article on the Soyuz–Apollo project which pointed out that the docking fault which led to the deaths of the three cosmonauts who visited the Salyut-1 station had been overcome, and Salyut-2 would in due course be visited by a crew aboard a Soyuz. No such visit took place, however, and on April 12, the traditional Soviet "Cosmonauts' Day" (the anniversary of Gagarin's flight), it was announced that Salyut-2 was carrying out its assigned mission—the testing under remote control of on-board systems. Observers reported, however, that one of the solar panels of Salyut-2 had become detached, so that clearly the station was no longer in working order for a manned visit. Later TASS announced that Salyut-2 had "completed its mission" satisfactorily.

When the Soyuz–Apollo mission was first suggested it was considered that the chief problem would be to decide on whether to use air at reduced pressure or oxygen as the atmosphere. The language barrier was also considered to present difficulties (see *Nature*, **239**, 362; 1972). According to Academician Petrov (*Pravda*, August 2, 1972) work on the mechanics of the link-up is already in progress. But doubtless the problems that Skylab has run into are occupying the Americans more at present.

NEW WORLD

AEC Promotes Safety Research

by our Washington Correspondent

AMID rumours of a palace revolt and Congressional displeasure, the Atomic Energy Commission is going ahead with a reorganization plan designed to give more prominence to research on reactor safety. The plan represents an abrupt *volte face* on the part of most of the Atomic Energy Commissioners and it may lead to the resignation of Milton Shaw, the powerful head of the AEC's Division of Reactor Development and Technology. But, on the other hand, it may help to head off criticisms that reactor safety has suffered from conflicts of interest within the agency, and it seems to represent a victory for the AEC's new chairman, Dr Dixie Lee Ray.

The essence of the reorganization plan is that safety research on water-cooled reactors is being taken out of Shaw's division and placed in a division of its own. Dr Herbert J. C. Kouts, a nuclear physicist at present employed at Brookhaven and a former chairman of the Advisory Committee on Reactor Safeguards, has been appointed director of the new division and will report directly to the AEC's General Manager. According to Dr Ray, the changes will "provide for greater emphasis and effectiveness in (the AEC's) safety research programmes", and they will "help to speed resolution of the still-unanswered questions in this rapidly developing technology".

Such a relatively minor bureaucratic reshuffle may seem an insignificant matter, but it represents a rather fundamental change of view within the commission, and it comes at a time when public concern over reactor safety is at its height. It seeks to redress a constant criticism of the AEC, namely, that it suffers from a conflict of interest that arises from its dual responsibility for promoting and regulating nuclear power. As far as safety is concerned, the agency's critics have suggested that since responsibility for safety research has been vested in the division which is also responsible for reactor development, it has not received the attention it deserves. The reorganization plan is thus an attempt to remove the appearance of conflict of interest and to elevate safety research to a higher position in the AEC's bureaucratic hierarchy.

But the reshuffle has not pleased everybody. Among those who are not enamoured with it are Milton Shaw, who has lost some of his responsibilities, Commissioner James Ramey and a few powerful members of the Joint Committee on Atomic Energy, the AEC's Congressional watchdog. They are concerned that since safety systems are such an integral part of reactor design, to divorce safety research from reactor development could be counterproductive.

But those who favoured splitting off safety research into a separate division —and Dr Ray was among them—argued that as far as water cooled reactors are concerned, development is essentially complete, and so there is no need to integrate safety research with development. The same cannot be said for the fast breeder reactors, however, and so the final reorganization plan keeps research on breeder reactor safety under Mr Shaw's wing.

The fact that a majority of the five-member commission eventually agreed to the reorganization plan represents a complete change of heart during the past year, for in April 1972 there was a move to shift responsibility for safety research to the AEC's Regulatory Division, but the commission was unanimously opposed to the idea. The Joint Committee on Atomic Energy in fact then held a day's closed hearings to discuss the matter with commission members, after which James Schlesinger, who was then AEC chairman, wrote to the committee to say that "removing the safety research function from the Division of Reactor Development and Technology would have detrimental effects upon the overall safety of reactors". Last autumn, however, Schlesinger proposed his own scheme to set up an office of safety analysis reporting directly to the commission, which would liaise between Shaw's division and the regulators. Commissioner Ramey said last week in a telephone interview that he still favours that scheme rather than the reorganization

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ENVIRONMENT

Out with a Bang?

by our Washington Correspondent

THE latest explosion in the AEC's Plowshare series shook a few buildings and raised some dust in Colorado last week, but it seems from preliminary reports that the blast triggered little damage and met with general apathy among local residents. Whether the test successfully achieved its objective of fracturing layers of rock deep under the surface to release trapped natural gas will not be known for several weeks, when a shaft will be redrilled to the cavern made by the explosion and the quantity of gas trapped there will be measured. Even if the test is an unqualified success, however, there are serious doubts about whether nuclear explosives will ever be used to exploit the vast deposits of natural gas trapped in tight formations beneath the Rocky Mountains (see *Nature*, **241**, 494 ; 1973).

The test, called Rio Blanco, ran into some opposition before it took place —environmentalists tried unsuccessfully to get it stopped by the courts, and Senator Floyd K. Haskell, the new Senator from Colorado, held a day of hearings into the project, but if the AEC and CER Geonuclear Corporation of Las Vegas, its industrial partner in the exercise, propose to take the project any further, they are likely to have to fight tooth and nail in the courts and to overcome some opposition in Congress and in other government agencies. The chief problem will be that, according to an estimate from the Lawrence Livermore Laboratory, it will take 370 blasts each year until the end of the century to exploit all the gas deposits underneath the Rockies—local residents may be willing to put up with one test shot, but not with several thousand.

The chief fears which have been voiced about the project are that the radioactivity from the blasts will seep into groundwaters and contaminate the Colorado River, but the AEC vigorously denies the possibility of that happening. The question has also been raised of the dangers that may arise from radioactivity in the released gas itself, but Dr Gerald W. Johnson, head of the Plowshare programme, told the Haskell hearings that a consumer would receive an annual dose of radioactivity from the gas about a hundred times smaller than that from a colour television set.

Rio Blanco itself was designed to test whether firing three explosives simultaneously would release more gas than a single shot—the test involved three 300-kiloton devices in a vertical line—and to test a low-yield explosive. The results will enable AEC officials to balance the possible risks associated with development of the gas field with the benefits of being able to double proven reserves of natural gas in the United States. There is a chance, however, that it may have been Plowshare's last blast.

now being put into effect because it would have left the Division of Reactor Development and Technology intact.

As for the Joint Committee, it met in closed session with Dr Ray last week to talk over the plan, and although no formal vote was taken, a majority of those present favoured the reshuffle. A few were not happy about it, however, and one of their chief concerns was that it may precipitate the resignation of Mr Shaw. A forceful administrator, Shaw is in charge of the development of the liquid metal fast breeder reactor, on which the Administration has pinned its hopes for the next generation of reactors, and some committee members were anxious that his possible resignation may hamper development of the breeder. Sources in the AEC and in Congress confirmed last week that Shaw is upset by the reorganization and at least toying with the idea of quitting.

What made the commissioners change their minds during the past year? A number of factors played a part, but the most influential was probably the public hearings last year on the emergency core cooling device (ECCS) which is supposed to flood the reactor core with water to prevent a melt down if the main cooling water were lost through a sudden pipe rupture. The hearings, which took place over more than a hundred days and which produced some 20,000 pages of oral testimony, were held because of doubts about the ECCS after test failures in 1971.

The public hearings brought to the surface a number of disturbing allegations that the results of safety research which casts doubt on the safety of reactors have been suppressed by AEC officials. It also became evident during the hearings that a number of scientists working for the AEC believe that the commission's standards are not strict enough. Whatever the merit of such allegations, the AEC moved a little towards the position of its critics last October when L. Manning Muntzing, the Director of Regulation, announced stricter operating criteria which may reduce the power output of some reactors, until doubts about their safety are cleared up.

Another factor in the commissioners' change of mind is that the Joint Committee will soon be holding its own hearings on nuclear safety. The hearings have been promised for the past six months, but the committee has been waiting for a comprehensive report on the matter from the AEC. The report is now reported to be in the final stages of revision, and should be delivered soon. Those hearings are likely to capture considerable public attention, and by moving now to head off charges of conflict of interest in safety research, the AEC may take some of the wind out of its critics' sails.

ENVIRONMENT

Voice in the Wilderness

by our Washington Correspondent

RUSSELL Train, Chairman of the Council on Environmental Quality, earlier this month lashed out at some segments of the oil industry and others who have made "the environment the whipping boy for our energy problems". Speaking at the American Power Conference in Chicago, Train criticized automobile and oil companies which recently conducted a massive advertising campaign charging that devices to control exhaust emissions from automobiles will increase fuel consumption. Such advertisements, Train pointed out, ignore the fact that comparable fuel losses come from the use of automatic transmissions and air conditioners, and that a vehicle weighing 5,000 pounds consumes twice as much fuel as one weighing 2,500 pounds—ten times the fuel loss from emissions controls. "Half truths are not going to help meet our energy needs," he said.

Train also sought to dismantle another common myth—that environmentalists have been responsible for delaying nuclear power plants. He said that of 75 plants now in various stages of construction, only about nine have been delayed by environmental challenges.

Moreover, in January of this year, he said, the Atomic Energy Commission had under review the operating licences for 35 nuclear power plants, and "with the possible exception of one or two units, the commission anticipates no delays solely on the basis of environmental considerations in the issuance of operating licences for these reactors". And environmentalists can hardly be blamed—as they often are—for the lack of refining capacity in the oil industry, Train suggested, because until the second half of last year, refineries in the United States were working at less than 85 per cent of their total capacity.

Train's remarks stand out like a sore thumb against statements of several other members of the Administration. Recently, for example, Earl Butz, Secretary of Agriculture, suggested that environmentalists should be the first to have their power shut off when energy supplies run low.

SEISMOLOGY

From NOAA to USGS

by our Washington Correspondent

THE Office of Management and Budget has finally given its approval for some of the seismology programmes and facilities of the National Oceanic and Atmospheric Administration (NOAA) to be transferred to the US Geological Survey. The move will probably take place in two phases, the first of which has been agreed and will take place on May 27, while the second is still under negotiation between officials of NOAA, OMB and the National Science Foundation. NOAA was forced to abandon much of its earthquake research because of cutbacks in its funds as part of President Nixon's drive to hold down federal expenditure (see *Nature*, **241**, 362; 1973).

The facilities involved in next week's transfer are those engaged in engineering seismology, earthquake prediction and earthquake hazards assessment, chief of which is the Earthquake Mechanisms Laboratory (EML) in San Francisco.

A spokesman for the laboratory said last week that the laboratory staff expects to carry on operations relatively unaffected by the transfer, and few harsh words have so far been spoken about the moves.

Phase two of the possible transfer has not, however, been agreed to by OMB, and it may raise some more opposition.

If it goes ahead, NOAA would lose responsibility for the World Wide Standard Seismograph Network (WWSSN), the National Earthquake Information Center in Boulder, Colorado, and all the other seismic observatories except those which are needed for the tsunami warning system. At present, NOAA has sufficient funds in its 1974 budget to fund the facilities involved in phase two of the transfer plan.

One concern is that seismic data should still be readily available to geophysicists under any system that is worked out.

Already tentative plans call for the National Earthquake Information Center to continue to provide raw data on the preliminary location of epicentres of seismic evens, while NOAA's Environmental Data Centers (which it will retain) will continue to provide more complete data. In other words, if the full plan goes ahead, the same facilities will continue to provide geophysical data as at present, but one will be under control of the USGS, while the other will remain with the National Oceanic and Atmospheric Administration.

One advantage of the scheme will be that planning and administration of the federal government's earthquake research will be under a single roof. In the past there has been considerable criticism of duplication of efforts and lack of cooperation between NOAA and USGS, and such problems should no longer arise once the new scheme is in operation.

NEWS AND VIEWS

Looking at the Hydrogen Economy

THE sudden recognition that an energy crisis is facing the world has loosed upon that same long-suffering world an avalanche of articles minatory, pontifical or plain gloating: heaven knows how much energy has been used in the production and absorption of these writings. Most of the prophets of doom are concerned only with present fuels and present modes of extraction and use, and very properly—given their premises—come to deeply pessimistic conclusions. A broader approach leads to less apocalyptic conclusions: thus Surrey and Bromley, in their contribution (*Futures*, **5**, 90 ; 1973) to the University of Sussex's analyses of the Club of Rome's *The Limits to Growth*, after concluding flatly that "there is simply no way of accurately assessing total world energy resources", rehearse the reasons for confidence that technological innovation can keep the crisis under control by developing new energy sources, new ways of using present ones more effectively, and ways of damping down the more profligate forms of energy waste.

Another voice of qualified optimism is that of Sir Alan Cottrell, the British Government's Chief Scientific Adviser. Last October he presented a survey lecture on materials and energy to members of the two principal American metallurgical societies, and this has now been published (*Metall. Trans.*, **4**, 405 ; 1973). His theme is the central part that constructional materials must play in the more efficient extraction and exploitation of fuels and in the more economical use of the resultant power: in the process, he covers with his usual clarity and verbal economy a very wide range of devices for extracting energy from fuels, and in particular focuses attention on some aspects which are usually neglected, especially techniques of storing energy. His article, most of which is accessible to non-scientists, should certainly be read by industrialists and publicists concerned with the future of energy.

In passing, Sir Alan repeatedly refers to the suggestions which have been made for the widespread use of hydrogen as a vehicle for converting heat into an easily transportable and storable form of energy. He quotes the electrochemist Bockris (*Science*, **176**, 1323 ; 1972) as one recent proponent of this notion: it has also recently been analysed in *Scientific American*. Water would be electrolysed at nuclear power stations, presumably at night at periods of low electricity demand, and the hydrogen distributed through the natural gas pipe network, refrigerated and stored in liquid form in giant Dewar vessels. One way of using it, as Cottrell points out, is for direct conversion into electricity at high efficiency by means of Bacon fuel-cells. A supplement to this proposal has recently been studied in the United States: liquid hydrogen proves to be an efficient and entirely pollution-free fuel for existing motor car engines, and a serious analyses of the advantages and problems of using it in this way is now in progress.

As outlined, the proposal for a "hydrogen economy" involves hydrogen merely as a convenient intermediary, plus a means of absorbing electricity at periods of low demand and thus improving return on capital. But hydrogen could in principle be used to better effect. Cottrell points out the low overall efficiency, even now, of heat engines and their attached electric generators, and the benefits which will attend really effective large-scale direct conversion of heat into electricity or other forms of expressed or stored energy. The production of hydrogen by electrolysis is not efficient: apart from the inescapable thermodynamic limitations on the conversion of "nuclear heat" into electricity, large industrial electrolysers, according to Marchetti (*Laser*, No. 34, 11 ; 1973), are only about 50% efficient and very capital intensive. Marchetti, who is in charge of materials research at the Euratom Laboratories at Ispra, Italy, makes this point in the course of a detailed account of the chemical hydrogen extraction process on which a team at Ispra has been working for some time. The Euratom method involves a cycle of several reactions. Several variants have been examined ; the mark I cycle is as follows:

$$CaBr_2+H_2O \xrightarrow{730^\circ\ C} Ca(OH)_2+2HBr$$

$$Hg+2HBr \xrightarrow{250^\circ\ C} HgBr_2+H_2$$

$$HgBr_2+Ca(OH)_2 \xrightarrow{200^\circ\ C} CaBr_2+HgO+H_2O$$

$$HgO \xrightarrow{600^\circ\ C} Hg+\tfrac{1}{2}O_2$$

The sum of these is: $H_2O \rightarrow H_2+\frac{1}{2}O_2$.

According to Marchetti, the thermodynamic efficiency of this cycle is about 85%, and the practical efficiency overall is at present about 55%, and still improving. Materials seem to pose no serious problems and recovery of the catalysts is straightforward. He goes on to a detailed analysis of the economics, in terms of capital costs and running costs assessed separately, of the generation and transport of hydrogen. The further implications of his proposals are to be discussed in the next issue of *Laser* (a scientific magazine published privately from 26 Selwood Road, Addiscombe, Croydon).

From information publicly available it would seem that at present all the running in this field is being made by American industry and by Euratom. It is to be hoped that the powers that be in Britain are at least maintaining a close watching brief at this stage.

The growing interest in the use of liquid hydrogen as a transportable, highly concentrated form of fuel can be regarded as a form of technology transfer. The American space programme has turned liquid hydrogen from an alarming speciality (used largely for nuclear bubble chamber research) into a familiar and not particularly dangerous commodity. But it is one thing for a large government agency to handle a material which was formerly thought unacceptably hazardous and quite another for the man in the street—or the roadside garage man—to do likewise. The precedents are not encouraging: liquid sodium is another material in the same category as liquid hydrogen. It has become familiar within specialized engineering circles because of its essential use as a coolant for certain types of nuclear reactor, and has been handled with complete safety for years past. Nevertheless, a brave attempt by a Union Carbide subsidiary to manufacture plasticsheathed sodium cables for the transport of electricity has failed and the factory has had to be closed.

From our Materials Science Correspondent

Synaptic Ghosts

THE arresting phrase "ghosts of childhood" was used by Merrill and Wall (*J. Physiol.*, **226**, 825 ; 1972) when they were discussing the presence of ineffective synaptic connexions in the spinal cord of adult cats. It had previously been widely assumed that the existence of a morphological synapse between two neurones indicated a functional connexion, either excitatory or inhibitory, between these two cells. Merrill and Wall, however, described synapses that were effective only if activated artificially, by electrical stimulation, and were not driven by natural stimuli. They suggested that these synapses may have been naturally active early in development, only subsequently losing their effectiveness.

Mark and his colleagues (*Brain Res.*, **19**, 41, 53 ; 1971 ; and **46**, 131, 149 ; 1972) had earlier described a related phenomenon after regeneration of the oculomotor and trochlear nerves in fish. The normal nerve supplying an eye muscle was cut and that muscle forced to receive innervation from a foreign nerve. Functional synapses were established between the foreign nerve and muscle. When the normal nerve regenerated into the muscle, not only did it establish functional synapses, but its arrival also repressed the activity in the previously functional synapses formed by the foreign nerve. This repression occurred very rapidly and in the absence of observable morphological changes in the repressed synapses.

On page 201 of this issue of *Nature*, Cass, Sutton and Mark report on the finding once again of ineffective synapses, in this case in the salamander limb. Electrical stimulation of a segmental nerve supplying the limb yielded muscle contraction and functional potentials within a well defined muscular territory. Section of that segmental nerve, however, produced degenerating synapses which extended beyond the functionally defined territory of that nerve. Conversely, within the territory were found undegenerated synapses deriving, presumably, from other segmental nerves, although electrical stimulation of these latter nerves did not produce synaptic activity within the territory. This non-functional innervation occupied a band about 0.5 mm wide on either side of the territorial boundary. These ineffective synapses in the salamander differ from those described in the cat in that they are unresponsive even to electrical stimulation.

The presence of such synapses raises two questions. What do they represent, and what is responsible for the repression of their activity? Cass *et al.* suggest that chemospecific developmental forces direct nerve fibres to approximately their natural muscle territories but this process is refined by a competition between terminals for the control of muscles. The authors are a little vague as to the nature of this competition implying, in one instance, that it may be based on selective chemospecific recognition between nerve and muscle cells and, in another, suggesting that relative amounts of impulse traffic in competing terminals may be the critical factor. The end result of the competitive process is not the destruction of the defeated synapses, but their functional repression, leaving a fringe of functionless synapses bracketing the territorial boundaries. The mechanism of the repression is not known, but the experimental situation indicates that it occurs at the neuromuscular synapse. The repression is not, however, an irreversible change occurring once and for all during development, but rather seems to be a dynamic on-going process. Function can reappear at these repressed synaptic sites.

The spinal nerve supplying a muscle territory was cut and the muscle was paralysed. Within 2 to 3 days, however, stimulation of adjacent nerve roots led to activity in the boundary fringe region. Cass *et al.* suggest that this early appearance of activity reflects the emergence of function in pre-existing but functionless nerve terminals. One to two weeks later, activity appeared in the remainder of the denervated muscle and this was attributed to collateral sprouting from fibres of adjacent nerve roots. Later, the original sectioned nerve regenerated back into the muscle, re-established functional synapses and suppressed all the foreign innervation. The suppressed synapses do not apparently disappear because cutting the normal nerve a second time resulted in the rapid derepression of foreign innervation ; in this case activity appeared within 2 to 3 days throughout the denervated muscle instead of just at the boundary fringe. This conclusion is plausible but should be confirmed by the demonstration that after regeneration of the normal nerve, section of an adjacent root produces degenerating synapses throughout the muscular territory of the normal nerve.

The concept that during development synaptic relationships are established more widely than is apparent from the adult pattern is not new—it was one of the principal thrusts of Cajal's speculation on neurogenesis. What is new is the notion that these excess synapses, or a proportion of them, survive in a morphologically normal, albeit functionally ineffective, form. The substantiation of this view would mean that one could no longer accept that the anatomical demonstration of a synaptic connexion is necessarily equivalent to the demonstration of a functionally effective pathway. The suggestion by both Merrill and Wall and Cass *et al.* that functional activity in competing pathways may determine the pattern of effective synapses seen in the adult animal deserves further investigation.

From a Correspondent

Foetal Antigens and Cancer

ENTHUSIASM for the notion that the growth of malignant cells is regulated by an immunological mechanism has, as Prehn has pointed out, waxed and waned over the years. Thomas initially put forward the idea that there might exist an immunological surveillance mechanism operative against potentially malignant cell populations and Burnet has popularized this theory. Experimental validation for it has been strangely slow to accumulate although, in recent years, the relatively high incidence of malignancies in certain groups of chronically immunosuppressed patients has been cited as evidence for the hypothesis. In spite of these uncertainties many attempts are presently being made to apply immunotherapeutic techniques in the treatment of various kinds of cancer, and the concept that malignant cell populations are antigenically different from the normal cells from which they derive is widely held to be useful.

One view is that cancer cells sometimes or always express antigens which are normally only to be found or are only found in large amounts in foetal tissues. Seen thus, malignancy is a form of dedifferentiation. The carcino-embryonic antigens and α-foetoproteins which are commonly found associated with certain kinds of cancer and which normally are characteristic of foetal tissues exemplify this point. The notion is attractive and is accumulating evidence. On page 225 of this issue of *Nature*, Castro and his colleagues report an experimental approach to the problem which might eventually show how an understanding of foetal antigens can be used in cancer therapy and perhaps even prophylaxis.

A mixed bag of foetal tissues was inserted under the kidney capsule of syngeneic or allogeneic recipient mice which were either normal or immunologically incompetent (by prior thymectomy, irradiation and bone-marrow injection). In normal mice neither syngeneic nor allogeneic foetal tissues grew well though syngeneic adult cells did. In the mice which were immunologically deprived foetal tissues of both kinds grew well and manifested differentiated cells of many sorts. Castro and his colleagues suggest that the failure of syngeneic foetal tissues to grow well in normal mice may indicate that there is a response against foetal antigens. Acting on this suggestion they went on to induce or transplant tumours in mice which had "rejected" syngeneic foetal tissue. In neither instance was a suppression of the malignancy observed; quite the contrary, the induction time was shortened and the growth of the transplanted tumour was enhanced. It might have been better had tumours which are known to express particular foetal antigens been used rather than chemically-induced tumours of ill-defined antigenicity. Nevertheless these studies do point the way for further and more specific experimentation.

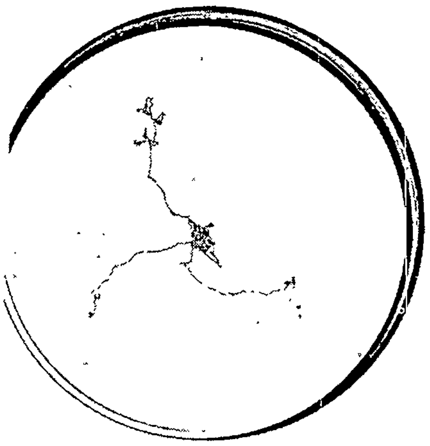
From a Correspondent

CHEMOTAXIS

Nematode Orientation

from a Correspondent

NEMATODES are largely studied as parasites in man, animals and plants and few critical data on their behaviour and sensory physiology are available. *Caenorhabditis elegans*, and a few other nematodes, are, however, becoming the focus of considerable attention as basic biological models. *C. elegans*, which is about 1 mm long, can be maintained in the laboratory on bacteria or in axenic culture. As with other nematodes, many tissues, including the nervous system, have constant numbers of cells, and there are less than 300 neurones in *C. elegans*. It has a generation time of a few days, is a protandrous hermaphrodite, can be mutated in ethylmethane sulphonate and the mutants can be stored in nitrogen.



Tracks of three wild type adult *C. elegans* responding to gradients of NH$_4$Cl in a thin layer of agarose. The worms were placed on the plate at the points marked by dots and were allowed to make tracks for 15 min. The gradient ranged from 50 mM in the centre of the plate to 0.05 at the edge, and it changed little during the time of tracking (from Ward, *Proc. US Nat. Acad. Sci.*, **70**, 819; 1973).

Ward (*Proc. US Nat. Acad. Sci.*, **70**, 817; 1973) believes that the study of behavioural mutants can lead to a correlation of behavioural alterations with underlying changes in anatomy, physiology and biochemistry. This will identify the neural circuitry mediating the behaviour and may reveal molecular mechanisms by which nerves operate. The ultimate correlation may be between single gene defects and specific patterns of behaviour.

Ward used the wild type and selected mutants of *C. elegans* to study its chemotactic behaviour and set out to identify the attractants, the location of receptors, and the mechanism of orientation. Chemical gradients were established in 'Sephadex' or agarose and the orientation to and accumulation within high concentrations of attractants did not differ between larval stages and adults. *C. elegans* moved towards cyclic AMP and cyclic GMP at initial concentrations of 0.2 to 3.2 mM, but were not attracted by 3′-CH$_2$ AMP, N^{6}O$^{2'}$-dibutyryl-cyclic AMP, 3′ AMP and 5′ AMP. Certain, but not all, anions and cations were attractive, as were OH$^-$ and some amino acids, but sugars were not. When cyclic AMP at 2.5 mM was distributed uniformly in 'Sephadex' before the establishment of a cyclic GMP gradient, normal orientation to cyclic GMP was eliminated. This suggests that both molecules act on the same receptor site. The presence of cyclic AMP did not affect orientation to Cl$^-$, and Cl$^-$ and Na$^+$ did not compete. At concentrations of 0.3 M and above, the salts were repellent.

When moving over 1.5% agarose, *C. elegans* inscribes tracks and detailed analyses of its orientation are possible (see figure). Ward found that wild type individuals swam in relatively straight lines up to the gradients, but outside gradients or in areas of uniformly high concentrations, they turned, spiralled and frequently reversed. A slow mutant with degenerate muscles oriented equally as well as the wild type, but took eight times as long, demonstrating that orientation is independent of the forward velocity. Ward concludes that orientation is determined by the lateral movement of the head, and is therefore by clinotaxis. Results of experiments on other mutants with cuticular "blisters" support the notion that the amphids are chemoreceptors, an assumption which is likely to lack electrophysiological support for some time. The sensory receptors on the tail, the phasmids, seem not to be involved in the orientation response.

POLYPEPTIDES

The Missing Magnitudes

from our Molecular Biology Correspondent

PERHAPS the best justification that nowadays remains for the study of synthetic polypeptides as a twig, if not a branch, of molecular biology is that they allow theories of the kinetics of conformational transitions to be put to the test, and that the analysis of the kinetics of rapid processes in general has an undoubted relevance to a variety of interesting biochemical systems. The theory of the order–disorder transition of polymers in solution has reached a state of considerable refinement, and the indications are that it can account rather well for almost all the available experimental facts. As regards the archetypal case, that of the α-helix-random coil transition in synthetic homopolymers, quantitative data are sparse, however, since relaxation times in the microsecond region are involved, which are not accessible by the temperature-jump or related techniques. With the use of the Ising lattice formulation, Schwarz calculated the kinetic characteristics of such transitions taking account of the existence of nucleation and propagation processes in the formation of ordered structure, and arrived at the expectation of a maximum relaxation time at the transition midpoint—which, the indications are, is nevertheless still in the submicrosecond range.

The general accord on the characteristics of the transition was breached only by the unexpected observation that proton magnetic resonance was able in certain cases to resolve contributions from α-carbon proteins in the α-helical and the randomly coiled states. Now this would imply slow interconversion between these conformations relative to the nuclear relaxation time scale, in other words a transition rate which is a round half-dozen orders of magnitude slower than it should be. Various explanations were offered for this phenomenon, of which only two survived closer scrutiny. One was that the polypeptide samples are all polydisperse, and because the longest chains form the most stable helices, and exhibit the sharpest transitions, one is simply resolving the signals from different size populations. The other suggestion was that the helix-nucleation step is slow in the polymer-solvent combinations in question, and the distribution of conformations at equilibrium is in fact very different from that calculated from the Ising lattice model.

This proposition has now been reexamined by Miller (*Macromolecules*, **6**, 100 ; 1973), who has calculated conformational lifetimes for more elaborate models. In particular he has examined the effect of the assumption, made for ease of computation, that the probability of finding more than one run of helix within a single chain is low. This he shows leads to an overestimate of the helix lifetime. The values for the lifetimes also depend on the balance of the thermodynamic parameters for helix formation and the chain length. By choosing a combination of values for the parameters in the model, Miller is able to simulate the observed NMR spectra. That the splitting of helix and coil contributions has been observed only in non-aqueous solvent systems can be easily enough rationalized on the basis of different values for the helix initiation parameter in the two cases.

It only partly detracts from the interest of this analysis that the NMR results which it seeks to explain are evidently in fact no more than a consequence of polydispersity. In the interim Nagayama and Wada (*Chem. Phys. Lett.*, **16**, 50 ; 1972) have examined very narrow cuts of polybenzylglutamate (with a weight to number average molecular weight ratio of 1.04), and shown that there is no α-carbon proton splitting in the helix-coil transition region, thereby, one may hope, laying the ghost once for all.

One of the few approaches to rates of conformational transitions in these short time ranges is the measurement of ultrasonic absorption. In aqueous polyglutamic acid a relaxation corresponding to the helix-random coil transition was observed, and showed a maximum at the *p*H of the transition midpoint, as demanded by Schwarz's theory. Other ultrasonic absorption results, however, were too hastily interpreted and proton transfer processes were wrongly taken for the helix-coil transition. Zana and Lang (*Biopolymers*, **12**, 79 ; 1973) have now done measurements on polybenzylaspartate in a mixed solvent system, and find that though the level of absorption is different in the α-helical and random states—presumably in consequence of solvation differences—no maximum of absorption, such as would correspond to the transition process itself, is to be observed in the working frequency range. This makes it possible to set a lower limit on the relaxation time of the transition. The corresponding rate constant is higher by at least an order of magnitude than that observed for polyglutamic acid in water.

The various limitations in all these methods should direct all the more attention to an entirely new and potentially very versatile approach to fast kinetics. Feher and Weissman (*Proc. US Nat. Acad. Sci.*, **70**, 870 ; 1973) have evolved a technique for the study of the concentration fluctuations which prevail in a reacting system at equilibrium. The beauty of this method is that the equilibrium is not disturbed in the course of observation. It has been tried out on a system in ionic dissociation equilibrium, using the solution conductivity as the vehicle of observation. Feher and Weissman have succeeded in separating true concentration fluctuation noise from electronic and other types of noise, and have extracted a frequency spectrum for the fluctuation. The temperature dependence yields a linear Arrhenius plot, and an activation energy agreeing precisely with the value from relaxation measurements. Inasmuch as conformational processes involving macroions, such as nucleic acids and proteins, are in general accompanied by changes in counterion binding and therefore solution conductivity, this approach may well be one of the brightest prospects now on the horizon.

INFLUENZA VIRUS

Human Pandemic Strains

from our Cell Biology Correspondent

IN 1968 the so-called Hong Kong strain of influenza virus arose in China and proceeded to cause a worldwide epidemic of influenza. The origin of Hong Kong influenza virus has remained something of a mystery, but two virologists, Laver and Webster, who have a bent towards molecular detective work, believe they may at last have uncovered the origin of this virulent virus (*Virology*, **51**, 383 ; 1973).

Last year Webster and Laver reported that the haemagglutinin subunits of the 1968 Hong Kong virus and preceding A2 Asian influenza viruses were completely different both in their immunology and their amino acid sequence. These observations made it highly unlikely that the Hong Kong virus was simply a mutant of some pre-existing human influenza virus. On the other hand, in 1968 and 1969 others had shown that the neuraminidase subunits of the Hong Kong virus were antigenically identical to those of preceding A2 Asian influenza viruses. These two

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Sequencing of Sticky Ends

MANY coliphages, the most familiar example being lambda phage, contain a double stranded genomic DNA molecule with what are known as cohesive or sticky ends. The two ends of such molecules are single stranded and mutually complementary in base sequence so that the linear molecules can form circles, the two single stranded tails base pairing with each other, or can polymerize to form a linear or circular concatamers. The base sequences of the sticky ends of the lambdoid phages are apparently similar because DNA of phages of this family form mixed dimers, the single stranded tail of one phage DNA base pairing with that of its relative. Likewise another family of temperate coliphages, which include phages 186, P2 and 299, show a similar relationship with each other but not with the lambdoid phages. Murray and Murray have directly confirmed these relationships by analysing the base sequences of the sticky ends of five lambdoid phages and three other temperate phages. They report their results in *Nature New Biology* next Wednesday (May 30).

Their painstaking analysis, which testifies to the considerable progress that is being made in the development of techniques for sequencing directly DNA molecules, proves that the cohesive ends of the five lambdoid phages studied have identical nucleotide sequences. The sequences of the sticky ends of phages 186, P2 and 299 by contrast are not identical but they are similar, and sufficiently so for the DNAs to cohere in spite of regions of mismatch. These data indicate that during the evolution of these phages the sequence of the sticky ends has been conserved no doubt because of the part they play in the life cycle of the phages.

sets of observations led Laver and Webster to the conclusion that the Hong Kong virus might have arisen by recombination between an existing human virus and an animal or avian virus ; the first donating neuraminidase subunits, the second donating haemagglutinin subunits to the recombinant Hong Kong virus.

In 1968 and 1969 it had also been shown that two strains of influenza virus that in 1963 had been isolated from ducks and horses were serologically related to the human Hong Kong virus and that this relationship was probably mediated only through the haemagglutinin subunits of the three viruses, avian, equine and human, because the neuraminidase subunits of the three proved to be quite unrelated.

What Laver and Webster have now shown is that the duck, equine and Hong Kong influenza viruses do indeed have haemagglutinin subunits that are serologically cross-reacting. Furthermore, by comparing peptide maps of the haemagglutinin subunits of the three viruses they have established that the light polypeptide chains of the subunits are virtually identical whereas the heavy chains differ significantly. It seems, therefore, that the Hong Kong virus might well have arisen by recombination between a human virus and an equine (or avian) virus related to equine 2 (or duck/Ukraine) influenza virus, and Webster and his colleagues have apparently obtained such human/animal (or avian) recombinants in experiments which mimic natural conditions of transmission.

Downie and Laver (*ibid.*, 259) also believe that there are probably plenty of as yet undetected avian influenza viruses in existence from which human pandemic strains may arise at any time as a result of such recombination. Indeed, they have isolated such a virus from an apparently healthy Australian shearwater. This avian virus is a type A virus, and of course pandemics of type A viruses occur in man. The haemagglutinin subunit of this shearwater virus belongs to a known avian type, but the neuraminidase subunit of the virus is antigenically distinct from all other known avian influenza viruses.

This virus is only the second type A influenza virus ever to have been isolated from a wild bird ; it did not apparently cause any disease in its host and it has a novel neuraminidase subunit. Presumably many wild birds harbour infections by benign influenza viruses and they probably provide a pool of viruses which by recombination with existing human viruses can give rise at any time to new virulent strains able to cause pandemics in man. In short, epidemics of influenza seem certain to continue to plague man for the foreseeable future.

ANIMAL BEHAVIOUR

Aggressive Anemones

from our Animal Behaviour Correspondent

AGGRESSIVENESS is not a trait very obviously associated with sea anemones. Yet Francis (*Biol. Bull.*, **144**, 73 ; 1973) has not only found overt aggression in the Californian anemone *Anthopleura elegantissima*, but also produces evidence that they are selectively aggressive to anemones of a different genotype from themselves.

A most striking feature of the spatial distribution of the anemones is that they live in clumps: there are definite patches of animals interspersed with anemone-free spaces. A given clump is made up of individuals all of the same colour pattern and of the same sex. The anemones are known to reproduce asexually by longitudinal fission, so that the different clumps are almost certainly groups of genetically identical individuals (clones). When taken into the laboratory and mixed together, the anemones segregate back into their clones.

Contact between genetically different individuals was found to lead to an elaborate series of aggressive behaviours, one or both animals being damaged in the process. First, the tentacles touch and withdraw repeatedly. If the anemones are from the same clone, the animals move toward each other, their tentacles interlacing. But if they are from different clones, this touching and withdrawing of tentacles is followed by distension of the acrorhagi — rounded objects situated beneath the outer layer of the tentacles. The inflated acrorhagi (now cone-shaped) are drawn upwards and then swept downwards towards the victim and wiped against its body. Tissue touched by the acrorhagi is damaged by the stinging cells they contain and begins to deteriorate. The victim either moves away or, if it is unable to do so, is killed.

Anthopleura shows this behaviour neither to species upon which it preys (for example, small mussels) nor to its predators (for example, sea slugs) and the curious clumped distribution of these anemones over the rocks would seem to be attributable to their own aggressive behaviour which has the effect of spacing them out from other anemones of alien genotype.

PLASMAS

Latest with Lasers

from a Correspondent

A CONFERENCE on laser produced plasmas, organized jointly by the Quantum Electronics and Plasma Physics Groups of the Institute of Physics, was held from April 17 to 18 at the University of Hull. The keen interest shown in the subject fully reflects the rapid increase in international activity since last year's publication of some

Shutoff Mechanism after Phage Infection

SHUTOFF of host transcription in *Escherichia coli* cells after phage T4 infection is prevented by chloramphenicol and it has been assumed therefore that a T4 protein(s) synthesized after infection is responsible for shutoff. But attempts to find the presumptive gene(s) which code(s) for such T4 protein(s) have been unsuccessful. The problem could be solved by study of a mutant defective in host shutoff. An RNA polymerase mutant (*Rif*^{R}-2) has been found which makes host RNA as late as 6–8 min after infection, and in next Wednesday's *Nature New Biology* (May 30), Snyder describes experiments with this mutant which cast a new light on the possible mechanism of host shutoff in phage infected cells.

Snyder investigated differences in sedimentation rate and efficiency of utilization of various templates in the RNA polymerase of infected and uninfected cells. These differences appear very early in wild type infection, but are delayed after infection of mutants in which shutoff of host transcription is also delayed. The effect is independent of chloramphenicol treatment.

The RNA polymerase from uninfected cells sediments as a broad peak in glycerol gradients. Different parts of the polymerase peak transcribe different templates with varying efficiency, showing that the polymerase peak is heterogeneous. In particular, a rapidly sedimenting polymerase activity disappears quickly after T4 infection and the polymerase peak becomes more homogeneous. In the *Rif*^{R}-2 mutant, however, the change in the profile of polymerase activity is delayed and even 8 min after phage infection the rapidly sedimenting activity can still be detected. This difference between the polymerase activity after infection of the mutant and wild type cells is independent of chloramphenicol treatment.

These experiments were carried out on crude preparations of RNA polymerase. Snyder suggests that the rapidly sedimenting polymerase may result from weakly bound factors which would be lost during the usual purification procedures and thus have not been detected previously. Thus he proposes that host shutoff is caused by inactivation of these factors which are required for transcription of host DNA *in vivo* but not *in vitro*.

American thermonuclear computations, which indicated that a laser fusion reactor is theoretically possible (see for example Nuckolls *et al.*, *Nature,* **239,** 139 ; 1972).

For many physicists, the computer dominated this meeting also. After a review of laser fusion calculations by Dr K. V. Roberts (UKAEA, Culham), Dr D. E. T. F. Ashby detailed recent results obtained with the Culham MEDUSA code. This spherical "one-dimensional" Lagrangian code neglects degeneracy and radiative reabsorption effects, but does include D–D, D–T and D–^{3}He thermonuclear reactions ; fusion neutrons are assumed to escape from the plasma, and charged reaction products deposit their energy locally. Assuming heat transport by electron conduction, this relatively simple model predicts thermonuclear gains of $\sim$55 when 140 kJ of 10.6 μm radiation is absorbed by a 250 μg DT pellet, a result in good agreement with the work of Clarke *et al.* (*Phys. Rev. Lett.*, **30**, 89 ; 1973).

There is, however, an upper limit to the power density which can be transported by collision-free electrons ; when this "free-streaming" limitation is introduced in an approximate manner to a previously optimized calculation, the thermonuclear yield is predicted to drop significantly. This new result is sensitive to wavelength, for it is postulated in the MEDUSA code that incident light not absorbed by inverse bremsstrahlung in the tenuous corona is subsequently absorbed at the critical density. (During the subsequent discussion it was suggested from the floor that the predicted decrease in yield could be alleviated by modifying the laser pulse shape to correct for the finite thermal transport time between the absorbing corona and the compressed core.)

Laser wavelength effects were also discussed by Dr G. J. Pert (University of Hull) ; "one-dimensional" numerical calculations indicate that of an incident 1.06 μm, 60 kJ laser pulse, only 1 kJ is absorbed by free–free absorption processes. Parametric and stimulated scattering processes are normally expected to absorb much of the remaining energy ; however, it was claimed that at very short wavelengths free–free absorption would be too weak to compensate for the higher threshold intensities expected for anomalous absorption. Finally, Drs J. A. Wesson and J. E. Crow (Culham) discussed model compression experiments, in which the principle of adiabatic compression by pulse shaping could be demonstrated and heat transport and laser-plasma interaction effects tested using currently available 1–10 GW CO$_2$ lasers.

Excellent reviews on the physics and technology of ultraviolet, CGE Nd-glass, and CO$_2$ lasers were given by Professor D. J. Bradley and Dr M. H. Key (University of Belfast), Dr J. Guyot (Laboratoire de Marcoussi) and Dr D. J. James (University of Hull). These talks provided a good introduction to the real world of laser plasma experimentation and gas breakdown phenomena, topics which occupied more than half of the contributions. An understanding of gas breakdown phenomena is of topical interest in the design of high power molecular gas lasers ; it is therefore noteworthy that Professor C. Grey Morgan (University College of Swansea) concluded his lucid review of this subject by observing that there is to date no unified theory which can account for all the existing CO$_2$ and Nd laser breakdown measurements. This observation was subsequently substantiated by new 3 ns CO$_2$ laser breakdown data (Dr G. Hill, University of Hull).

The meeting concluded with a contribution from Dr I. J. Spalding (Culham) on the reactor implications of laser fusion. Assuming electrical excitation, lasers having a pumping efficiency of some 15–30% and capable of generating thermonuclear gains exceeding 30, are required for economically attractive reactor applications.

Overall, the meeting left one with the impression of a wide range of interest and of expertise within British universities and research establishments. Many relevant experiments are possible with laser energies of 0.1–10 kJ and intensities of 10^{10}–10^{16} W cm^{-2}.

REMOTE SENSING

Earth Gazing

from a Correspondent

THE first Earth Resources Satellite is in space, orbiting tropical and temperate latitudes and systematically recording by remote sensing systems details of the Earth's surface. Some of the instruments, problems, costs and benefits of obtaining, analysing and applying remote sensed imagery were discussed at a three-day symposium held at University College, London, between April 10 and 12 and sponsored by the British Interplanetary Society.

The symposium was less confined than the title would suggest, as many of the contributions and much of the discussion dealt with Earth observation platforms other than satellites. Several contributions were concerned with the use of the British Skylark rocket as an airborne platform for remote sensing. Dr N. Simmons (Ministry of Defence) assessed the relative merits of various airborne platforms, principally aircraft, rocket and satellite, and then dealt in detail with the particular merits of the operational Skylark rocket system.

Several contributions dealt with sensor technology, some with instrumentation and others with management and development problems and proposals. Dr O. Hofmann (Messerschmitt-Bolkow) outlined a new and quite novel multispectral vidicon scanner

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Simple Models for Intramolecular Interactions

INTERACTIONS between acidic proteins and nucleic acids occur in a variety of interesting biological circumstances but the details are little understood. Novak and Dohnal (see *Nature New Biology* next Wednesday, May 30) have used the tactic of investigating the interactions of simple model compounds.

Starting with the supposition, for which there is growing experimental evidence, that strong interactions between purines and aromatic amino acids can occur, Novak and Dohnal manufactured the pentapeptide TGTGT where T is tyrosine and G glycine. This compound, they found, increased the T_m of calf thymus DNA by 6°, an amount significantly larger than that produced by basic peptides such as Arg$_4$. Moreover, the DNA peptide mixture was hyperchromic and this, they suggest, may result from the tyrosyl chromophores being introduced into a hydrophobic environment.

In support of this Novak and Dohnal found that the absorbance at 265 nm of TGTGT doubled when the peptide was transferred from water to dimethylsulphoxide. By contrast, they found that the analogous peptides TProTProT, in which rotation about the C–N bond is impeded, and the acidic peptide TGluTGluT had no effect on the T_m of DNA.

The effect of TGTGT was not confined to DNA, for it also stabilized poly(rA)·poly (rU) and poly(dG)·poly (dC) which have secondary structures differing from that of DNA ; by contrast, the peptide did not stabilize poly(dA–T)·poly(dA–T) or poly(dA)·poly(dT). These observations clearly do not admit of a simple explanation for the specificity of the stabilization, but some specificity for polymers containing G–C received support from precipitation experiments. Dinucleosides containing G were quantitatively precipitated by TGTGT at a ratio of one peptide to one dinucleoside. Dinucleosides which do not contain G were not precipitated.

Further experiments showed shifts in the NMR spectral lines corresponding to protons in both TGTGT and poly(A) in a mixture of these, and these shifts were all consistent with the tyrosyl residues intercalating between the purines—presumably thus placing themselves in a hydrophobic environment. This notion has obvious attractions, and it will be interesting to see if this proposed mechanism can account for interactions in real biological systems.

which operates without any moving parts—a great asset for remote operation.

Mr J. Plevin (Space Application Division, ESRO) presented a very reasoned case for the provision of an aircraft facility with advanced sensors, for European Earth resource scientists, in preparation for a European satellite programme. Even if a European satellite does not materialize there is still an urgent need to provide these facilities for the research scientists and in view of the costs involved the idea of a "European" facility is most rational and economic.

Mr S. L. Entres (Royal Aircraft Establishment, Farnborough) presented a masterly paper on the applications of orbital remote sensing in which he illustrated the efficiency of the system for the rapid collection of huge masses of information and showed that even at "pilot project", "evaluation" levels people are being drowned in data. He stressed that much more effort must be devoted to data handling and analysis, especially in developing automated systems. This, he argues, should take priority in current and near future remote sensing activity. Professor J. Hannessian (National Science Foundation, Washington) generated considerable interest with his contribution on the international and legal aspects of Earth observation satellites. Aircraft surveys over "alien" territory clearly violate national boundaries, but how high does a remote sensing platform have to be before it can be considered outside national boundaries.

The aim in space and airborne remote sensing operations is, of course, to apply the information collected to a specific problem. The "applications" groups were well represented and were drawn from a wide range of earth and environmental sciences. Mr N. P. Press (Royal School of Mines) spoke about the detection of the toxic effect of metals on vegetation and exploded the widely held (and printed) myth of the relationship between plant disease and chlorophyll content in the analysis of false colour photography.

Two contributions dealt with the problems of producing maps from satellite imagery. Dr O. Kolbl (Swiss Forest Research) was concerned with improving existing maps by the combined restitution of aerial and satellite photographs, and Mr P. G. Mott (Hunting Surveys) presented the viewpoint of the photogrammetrist that enough ground control exists, or could easily be obtained, to permit small scale mapping of the unmapped territories of the world.

Atmospheric pollution at local, national and international levels is a serious and growing problem and there is an urgent need to develop efficient recording and monitoring systems. In this context Dr A. R. Barringer (Barringer Research Ltd) is one of the world's pioneers and he gave a fascinating account of some of the instruments and techniques he is developing for the remote measuring of certain types of atmospheric pollution.

The applications of remote sensing in climatology, vegetation, geology and hydrology were also considered and to illustrate further the broad interdisciplinary nature of the topic Dr W. G. Collins (University of Aston) outlined some of the work which is being carried out in the Remote Sensing Unit where the nine research students are drawn from seven different disciplines.

The symposium highlighted, and several contributors referred to, some of the most glaring defects in the remote sensing activities in Britain: its excessive fragmentation, poor communication and lack of any focal point.

Remote sensing involves instrumentation development, software development and applications, each of which, particularly the latter, cuts across the traditional discipline boundaries. This has resulted in a fragmentation of remote sensing activities in all agencies —universities, research councils, government departments and learned societies. Universities can do little by themselves (although the University of Aston has made a promising start) and although the research councils claim an interest in interdisciplinary research this has not been reflected in their support of interdisciplinary remote sensing research.

GEOPHYSICS

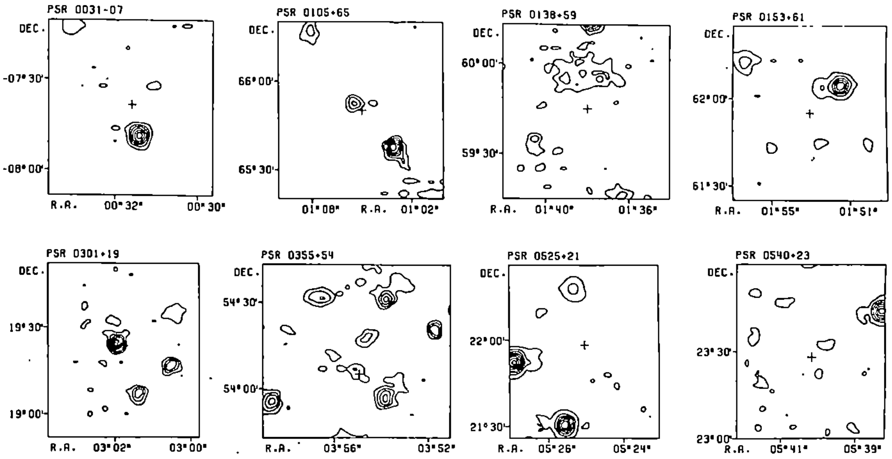
Volcanomagnetism

from our Geomagnetism Correspondent

OF the many potential methods of predicting seismic and volcanic activity, few can have proved more promising in theory and yet more discouraging in practice than that based on tectonomagnetism. It is now well established that when a stress is applied to, or removed from, a rock a small change in the remanent magnetization of the rock arises from the distortion of crystal structure (the piezomagnetic effect). Seismic and volcanic events reflecting changes in subsurface stress within the Earth should therefore manifest themselves as changes in the small part of the local geomagnetic field arising from the permanent magnetism in rocks. Continuous monitoring of the field should then lead to the observation of geomagnetic variations which reflect premonitory stress changes and thus possibly to a prediction of the time and location of an event.

Unfortunately, the phenomenon expected on the basis of theory and laboratory experiment is not easily observed in the field. For one thing, until recently, there have been practical problems associated with the measurement of very small field changes, distinguishing between the piezomagnetic signal and the much greater geomagnetic noise, and the insulation of magnetometers from mechanical vibration.

Continuum Radio Emission from Vicinity of Pulsars



THE suggestion that pulsars might possess weak radio haloes has been investigated by Schönardt (see next Monday's *Nature Physical Science*, May 28). His results are disappointing, in that they show no evidence to support the hypothesis, recently put forward by Blandford, Ostriker, Pacini and Rees (*Astron. Astrophys.*, **23**, 145; 1973). But they provide an important contribution to the general pool of knowledge about pulsar properties.

In this figure, eight of the nineteen radio maps prepared by Schönhardt are shown. Each map covers about one square degree, centred roughly on the appropriate pulsar. Measurements were made with the Max-Planck 100 m, equipped with an 11 cm cooled parametric amplifier; contour intervals vary from one pulsar to another, but these eight examples are typical in showing no shell sources associated with the pulsars.

Thus although correlations between geomagnetic changes and seismic activity have been reported quite frequently since 1799 (von Humboldt), few, if any, can be considered genuine. The problems of sensitivity and vibration have now been largely overcome in modern equipment; and noise from distant sources such as the ionosphere can be filtered out by coupling local magnetometers with reference stations away from the zone of local tectonic activity. Even so, few valid examples of geomagnetic change related to stress have been reported, although the expected magnitude of the effect is as high as a few gamma.

Perhaps the best known success is the correlation between local magnetic changes and creep events on the San Andreas fault observed by Breiner (thesis, Stanford University ; 1967), even though, at the same time, he failed to detect any relationship between geomagnetic variations and earthquakes in the same region. More recently, Davis and Stacey (*Nature,* **240,** 348 ; 1972) have further demonstrated that "the piezomagnetic effect is observable on a tectonic scale" by observing geomagnetic changes associated with the increasing water load in the Talbingo Dam of south-east Australia. Convincing observations of local magnetic changes preceding and accompanying eruptions of the Ruapehu and Ngauruhoe volcanoes of New Zealand have also been reported by Johnston and Stacey (*J. Geophys. Res.,* **74,** 6541 ; 1969, and *Nature,* **224,** 1289 ; 1969). On the other hand, Yokoyama (*The Surveillance and Prediction of Volcanic Activity,* UNESCO ; 1971) has attributed geomagnetic changes associated with Japanese volcanoes to local changes of temperature rather than to stress.

In an attempt to find further examples of the volcanomagnetic effect, Davis *et al.* (*Science,* **180,** 73 ; 1973) have investigated Kilauea Volcano in Hawaii. On the face of it, Kilauea offers a good opportunity for this type of work because it frequently erupts, highly magnetic basalts surround it, and the existence of the Hawaiian Volcano Observatory close by gives a good "logistic basis" for an experiment involving repeated observations of an instrumental array. Davis and his colleagues thus constructed a linked array of three total field magnetometers comprising one local station close to Kilauea and two more distant reference stations (10 km and 43 km away, respectively).

Recording of the geomagnetic field was continued for a (broken) period of 12 months in conjunction with the existing programme of tilt and strain measurement in Kilauea which has already demonstrated inflation of the summit before eruptions and deflation during flank eruptions. The geomagnetic changes to be expected from such tilts can easily be estimated. Thus a tilt of about 10^{-4} rad, implying a strain of about 10^{-4}, would give a stress of about 30 bar in basalt, assuming perfect elasticity. Titanomagnetite of the composition indicated by the relevant Curie points would then give an expected change in magnetization of 2 parts in 10^4 per bar of stress, which corresponds to an absolute change of at least 3×10^{-5} e.m.u. and a resulting magnetic anomaly of the order of 10 gamma if the basalt is magnetic to a substantial depth.

But although such magnetic changes were observed in one of the experiments, they failed to correlate directly with the tilt records, and in a second experiment, no magnetic anomalies developed at all. Thus when the local station was at Uwekahuma, close to the Hawaiian Volcano Observatory, brief excursions of the magnetic field occurred. These have a very slight correlation with small irregularities in the tilt records, but the effect is very localized. When the local station was moved to a site closer to the source of deformation, no magnetic anomalies developed at all even when tilting was violent.

The search for the volcanomagnetic effect from Kilauea was thus unrewarding, although even this negative result leads to a definite conclusion. It is clear that during a prolonged deformation of Kilauea the volcano cannot support any large scale pattern of shear stress but only transient, localized stresses accompanying local readjustments. Davis *et al.* do not discuss the effects of temperature at all ; but it is interesting to note that their conclusion based on experiment was also reached by Yokoyama who argued the general case based on a consideration of temperature.

Scattering in the Lower Mantle

IRREGULAR structures acting as seismic wave scatterers in the Earth are generally difficult, if not impossible, to detect because the small amount of scattered energy is easily obscured by the primary wave. But in seismic shadow zones the conditions are more favourable. For example, in the PKP shadow zone caused by the presence of the Earth's core, the first phase to be expected on the basis of the most simple core model is PKIKP, the phase refracted through the inner core. In practice, however, precursors to PKIKP are observed in the approximate epicentral distance range 125° to 143°—a phenomenon for which an explanation is required.

In fact, there are several possible (though not mutually exclusive) explanations, including more complicated core models energy diffracted from the PKP caustic surface in a limited range near the caustic, scattering from the Bullen D'' layer and in other parts of the mantle near the caustic surface, and scattering in the lower mantle beneath the source region. In next Monday's *Nature Physical Science* (May 28), Doornbos and Vlaar present an analysis of precursor data from the Norwegian seismic array (NORSAR) which provides evidence for all of these explanations except that involving a more complex core structure. In addition, they have been able to determine the locations of some of the inferred scattering zones.

From a spectral analysis of events in the Solomon and Fiji Islands regions during 1971 and 1972, Doornbos and Vlaar have been able to divide the precursors into three distinct classes based on differences in slowness ($dT/d\Delta$) and the azimuth of the direction of wave propagation. The first set comprises first arrivals in the range 143°–138° which have an average $dT/d\Delta$ value of about 3.3 s per degree and deviation from the true azimuth of less than 2° ; the second set includes phases with anomalously low $dT/d\Delta$ values in the range 1.6–3.0 s per degree and large azimuthal deviations in the range 10° to 40° ; and the third set consists of phases with anomalously high $dT/d\Delta$ values between 3.4 and 4.0 s per degree but azimuthal deviations within 5°.

There seems no reason to doubt that the precursors in the first set, at least those between 143° and 140°, are due to diffraction from the caustic as predicted by theory. Neither diffraction nor core complexity is, however, adequate to explain the second and third sets. If the phases in the second set are taken to be generated by scattering near the caustic surface, their characteristics may be used to determine the positions of the proposed scattering regions. Thus Doornbos and Vlaar conclude that scattering structures exist in the lower mantle at distances of 250 km, 500 km and 600 km from the core-mantle boundary. The errors on these locations are difficult to estimate, but are unlikely to exceed 100 km for the first one and 150 km for the other two. The uncertainties are thus not small but neither are they large enough to explain the depth spread of the locations.

The large $dT/d\Delta$ values for the precursors in the third set rule out an origin on the receiver side of the mantle but may be attributed to scattering structures on the source side. Accordingly, Doornbos and Vlaar also locate structures at distances of 200 km, 800 km and 900 km from the core-mantle boundary although in these cases the estimated errors amount to several hundred kilometres.

GEOPHYSICS

Mantle Plumes Get Cool Reception

"I DON'T believe them either, Harold," was how Professor Keith Runcorn comforted an incredulous Sir Harold Jeffreys at last week's meeting of the Royal Astronomical Society devoted to mantle plumes. Their scepticism was endorsed by almost all the attendance. The consensus was that there is little evidence for any of the mantle plumes which have been postulated to account for geological features on various parts of the Earth's surface, with the possible exception of the plumes held responsible for the Hawaiian islands and chains of seamounts in the Pacific.

Only one of the participants was more venturesome. Dr N. Petersen (Institute of Applied Geophysics, Munich) described work carried out with colleagues at Princeton University (see R. A. Duncan, N. Petersen and R. B. Hargraves, *Nature*, **239**, 82; 1972) as a result of which they identify two plumes at work on the European plate. The basic notion is of course that there are hotspots in the mantle which give rise to volcanic effects on the Earth's surface as the lithosphere moves over them, and it is an essential part of the hypothesis that the hotspots are comparatively narrow convection plumes. Apart from being a convenient way of accounting for the chains of volcanic features that litter the Earth's surface, these mantle plumes could conceivably initiate rifting and provide a mechanism for driving the plates. And if the hotspots can be considered fixed in the mantle—a qualification that may be difficult to sustain—then it ought to be possible to chart the movement of the plates by following the paths of the plume traces.

The two volcanic chains in the European plate which Dr Petersen and his colleagues suggest may be plume traces run in one case from the western part of the Eifel region in Germany through central Germany and Czechoslovakia to volcanic areas near Wroclaw in Poland, and in the other case from Iceland to the Faeroe Islands, the islands of the west coast of Scotland, the Antrim plateau and the Mourne mountains of Ireland. The youngest ends of the two traces are at Iceland and the Eifel region, and Dr Petersen and his colleagues claim that the motions of the European plate deduced from each trace are consistent.

Criticism of this work centred on the Iceland plume, possibly because a decreasing age of the igneous activity from east to west in the central European chain is demonstrated only when the times of the most abundant and intense eruptions are considered. When the total duration of activity at volcanic centres along the chain is examined the evidence for a migration of activity along the chain looks much less secure.

The chief point was that the evidence for migration of the Iceland plume along the section from the Mourne mountains to the Faeroes depends heavily on ages for the Mourne mountains and Antrim plateau which have recently been revised downwards so that they more nearly match the ages of Arran, Mull, Ardnamurchan, Skye, St Kilda and the Faeroes. This age problem becomes more serious if the island of Lundy in the Bristol Channel is considered to be the end point of the chain. There is also the problem of explaining several igneous regions further out in the Atlantic such as Rockall which are well away from the postulated trace.

Dr Petersen's reply was to admit that the age relationships are not as good as he would have liked, but he continued to remain faithful to the Faeroes–Iceland sector of the Iceland plume and to the central European plume.

Having devoured Dr Petersen, the meeting turned to a consideration of how the required hotspots might arise. Professor B. A. Bolt (University of California, Berkeley) described how seismologists are beginning to be able to study the nature of the boundary between the mantle and the core, the interest here being the possibility mentioned earlier in the meeting by Professor R. Hide (Meteorological Office) that bumps on the boundary could give rise to localized convective motions. Professor J. A. Jacobs (University of Alberta) described recordings by arrays of seismometers in Canada of earthquakes in the Tonga arc, the recordings suggesting the presence of inhomogeneities in the mantle beneath the Hawaiian islands. But Professor Runcorn (University of Newcastle upon Tyne) argued that the convection pattern from a hotspot on the boundary between the mantle and the core would not remain narrow but would spread out well before it reaches the surface. He felt that many people were imagining mantle plumes to be analogous to the narrow plumes of smoke which can develop in the atmosphere, whereas in fact the atmosphere is not a good model for behaviour in the mantle.

All the same, the meeting seemed prepared to concede that some kind of hotspot is responsible for the Hawaiian chain, even though there seems to be no firm evidence of mantle plumes at work on continental plates. But Professor D. L. Turcotte (Cornell University) cast doubt even on that. He suggested that the Hawaiian islands may be explicable in terms of a tensional fracture propagating in the lithosphere, causing volcanic activity as it migrates. The meeting on mantle plumes ended with Professor Jacobs's comment "We may be worrying about something that doesn't exist".

From Quantum Crystals to Neutron Stars

IT is now generally accepted that pulsars—or at least most pulsars—are rotating magnetic neutron stars. It is therefore of interest to study the structure of neutron stars, and this raises problems in several completely different fields of physics. First of all, nuclear physics should ideally provide an equation of state, but nuclear forces are as yet not understood with sufficient accuracy to lead to a really reliable equation of state. Second, elementary particle physics should reveal something about the state of matter at densities which are so high that apart from neutrons, protons and electrons, other particles such as hyperons will appear. Again present knowledge about elementary particles is still far from complete, so that part of the theory of neutron star interiors is still chiefly a speculation. Finally, there are several problems which fall in what is broadly called solid state physics.

In early studies of the interior or neutron stars it was generally assumed that it would be a fluid—which might be both superfluid and superconducting. Recently several authors have suggested that the interior of a neutron star may be solid. One such calculation is reported by Canuto and Chitre in *Nature Physical Science* next Monday (May 28). These authors apply a method, successfully used in describing quantum crystals such as ^{3}He and ^{4}He, and find that for densities above about 1.5×10^{15} g cm^{-3} the stable configuration is a face-centred cubic one in which the neutron spins show an "antiferromagnetic" ordering.

It is interesting to note that there is possibly some observational evidence in favour of a solid core. This evidence is provided by so-called glitches, the speedups of the Crab and the Vela pulsars, and the subsequent healing. These glitches have been explained in terms of star-quakes in which the interior of the neutron stars adjusts itself to the lower rotational velocity. Calculations by Pines, Shaham, and Ruderman (*Nature*, **237**, 83; 1972) have shown that to explain the magnitude and the frequency of the Vela glitches, it seems necessary to assume the existence of a solid core; this lends some degree of credibility to the results of calculations such as the one reported by Canuto and Chitre.

Metallic Ore Deposits and Continental Drift

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The application of plate tectonic concepts shows that ores associated with igneous activity must be concentrated in specific areas and allows determination of potential ore reserves from simple considerations.

THE history of the breakup of the continents during the past 200 m.y. is becoming increasingly clear[1,2] and obviously pre-existing ore deposits which have been separated during this time can be matched across newly formed oceans[3-5]. This is significant in locating unknown reserves, for example in Antarctica[6], but also means that techniques of exploration and exploitation which have proved most effective in one continent will also be the most efficient in matching regions. Locally such matching may be of direct economic importance, such as the predication of Pliocene copper ores in Mexico to match those of Boléo (Baja California) after reclosing the Gulf of California. Nonetheless, such matching is not of fundamental importance and becomes increasingly difficult for earlier times when the continental plates and metal provinces are more difficult to define.

Early concepts of the genesis of ores associated with igneous rocks were based on the assumption that metals segregated into the upper mantle and lower crust during the early cooling of the Earth and were remobilized and concentrated at the surface either by precipitation from waters heated by igneous intrusions or by direct magmatic differentiation. Clearly, any predication of potential reserves on this basis was largely empirical but at various levels of sophistication[7]. It had, for some time, been realized that certain ores were associated with particular geosynclinal conditions—although for unknown reasons. Recent studies of ore genesis where oceanic plates are being carried into the mantle along subduction zones[8-11], and where new oceanic crust is forming at the active oceanic ridges[12], allow the formation of ores to be considered throughout geological time in three principal environments—deep oceanic, subduction zone and stable continental block. As these environments have drastically changed their relative positions during at least the past 2,700 m.y., the determination of past continental relationships is fundamental to considerations of past ore formation. The past position of a continent relative to the Earth's axis of rotation can be obtained for any given time using palaeomagnetic methods[13], and similarly the relative positions of different continental blocks can be obtained if sufficient palaeomagnetic data are available for polar wandering paths to be compared—diverging paths indicating the blocks were then separated by a spreading ocean, converging paths requiring intervening subduction. These types of data also afford direct measurement of the palaeolatitude of the sampling sites, which is relevant to the formation of certain deposits.

Deep Ocean Environments

Mining of manganese-rich nodules which are strewn over the ocean floors[14,15] may become economic in the near future, but their growth processes are not understood, so it is not clear how different plate environments on the ocean floor could influence their growth and composition. Near the active oceanic ridges, however, the sediments are locally enriched in metals[16,17], particularly copper and molybdenum, in areas marked by high heat flows. Similar enrichments have also been reported in older deep sea sediments[18] formed presumably before these sediments were carried away from similar active parts on the flanks of the ridge, yet the volcanic activity at mid-oceanic islands is not accompanied by significant mineralization. This suggests that differentiation to give fluids rich in metals does not take place until after new oceanic crust has been carried away from the active magma injection zone. The localized high heat flows therefore mark areas where hot ore fluids are making contact with seawater in the overlying sediments with the resultant precipitation of copper sulphide, in particular, in a similar way to the genesis of the Kuroko copper sulphides of Japan[19]. This is presumably the way in which the massive pod and sheet copper sulphides of Cyprus were created[20,21] in the Troodos Massif when it was part of an ocean floor, as these deposits are strongly concentrated towards the top of the oceanic igneous rocks. "Oceanic" ore deposits may also include podiform nickel, chrome, and so on[22], formed by differentiation within the mantle and oceanic crustal rocks, or asbestos and laterites may form as a result of later chemical activity respectively before or after these rocks have been incorporated into a continent, such as during collision between different continental blocks.

The direct relationship between seafloor spreading and ore genesis was first clearly established in the Red Sea[23-25] where hot, highly saline brines are presently depositing a variety of metal minerals as they mix with colder waters. Early isotopic analyses seemed to indicate that these metals were chiefly derived from the surrounding rocks by the leaching effect of brines heated by newly forming oceanic crust in the Red Sea, but some isotopic evidence[26] suggests the metals may be derived from the mantle. This is of academic importance but, irrespective of the source, similar deposition would exist along any heated continental edge during initial separation. Most deposits formed during the past 200 m.y. have been covered by later sediments and are still covered by water. But some areas outcrop on land, for example, along the Nigerian Benue Trough which seems to have opened as the South Atlantic was opening[27], and mineralization along these areas may reflect deposits of the type found in the Red Sea.

Subduction Zone Environments

The successive occurrence of manganese, iron, porphyry coppers, gold, lead, zinc, silver and so on with igneous activity at increasing distances from oceanic trenches has been detailed, particularly for copper[8-10,28,29], in the Andes, Japan and the Philippines. The metals and minerals within the oceanic lithosphere carried down at subduction zones become molten at different temperature/pressure levels and therefore the composition of the magmas ascending to the surface from different levels varies away from the oceanic trench in both their major and minor elements[30-33] and their accompanying hydrothermal fluids. Similarly the age of the magmas varies according to their fluidity, the depth of overburden and the availability of channels along which the magmas and fluids can ascend. Because the oceanic lithosphere thickness is remarkably uniform, the quantity of magma released, and therefore metals, is related directly to the rate of subduction. But the ability of these melts to reach the surface will be strongly controlled by the relationship between the fracture pattern within the overlying continental rocks and the direction of motion of the continental plate. There is a correlation between the location of orogenic episodes in the western United States with changes in the direction of motion of the North American plate[34] and these changes also correspond to times of peak mineralization[35,36]. Further localization of volcanic, magmatic and metallogenic activity is controlled by the rare but thick aseismic oceanic ridges which, where they are being subducted, create compressive force on the overlying continental plate which closes the fracture pattern, preventing material rising to the surface from the subduction zone, particularly from depths less than 200 km. A notable example is where mineralization zones are restricted to the north and south along the borders of the Peru-Chile Trench where it intersects with aseismic ridges. The position of past intersections would vary with the direction of movement of the plates and the orientation of the ridge relative to the subduction zone. Such explanations are not exclusive of other restrictive factors. For example, the local geology into which the ore fluids intrude is significant in providing suitable physical and chemical environments in which the metal salts can be deposited. South American lead and zinc sulphides, specifically, tend to be locally restricted to areas where carbonate sediments are present[28].

This model of ore genesis can only be regarded as a first approximation in any given region because metallogenic provinces associated with subduction are complex, varying, for example, with the angle of descent of the oceanic lithosphere, which produces polymetallic areas where it is steep. Further complications arise where several subduction zones are simultaneously active within a single region, as in the West Pacific today, because these deposits may eventually become intermingled by the closure of marginal basins. Similarly, complexity can arise from repeated subduction at different times in the same locality, as in the case of Japan. Even greater complications may arise when previous subduction zones override a spreading ocean ridge, as in the western United States. Nonetheless, most metallogenic provinces along subduction zones of the past 200 m.y. or so can be explained by these fairly simple concepts and thus areas of ore genesis can be located by using, in particular, the palaeomagnetism of the ocean floors to measure changes in the direction and rate of motion of different plates. It is unlikely that the identification of much older subduction zones will be of such direct importance, as these orogenic areas have been subjected to erosion and the metals, often only economically concentrated in the top 2–3 km, have mostly been removed, leaving the generally less economic root zones. But the location of previous subduction zones is of major significance, for they are probable source areas for some important sedimentary ore deposits.

Stable Continental Block Environments

Present plate theories suggest that away from plate margins the continental blocks behave as passive environments on which only sedimentation and erosion could take place, together with some vertical movements in reponse to mantle and lower crustal flow, for example during isostatic crustal readjustment. On this model, therefore, these environments are free from igneous activity, except for the carbonatites derived from the mantle. The carbonatites[37-39], which include diamondiferous kimberlites, originate (by unknown mantle processes but seemingly related to continental separation) below the continental lithosphere, which is some 120 km thick by comparison with the 60–70 km thick oceanic lithosphere. The carbonatites are driven to the surface by an explosive blast of volatiles and, because of the accompanying degassing, contribute virtually no heat to the continental blocks. The formation of new ores in the stable block is, therefore, chiefly restricted to the redistribution and concentration of pre-existing ores. Archaean processes of greenstone/granulite segregations, or metals derived or generated by meteorite impact, are relevant sources, but I will not consider them here because plate tectonic concepts are not applicable to their origin.

Chemical erosion can remove soluble minerals, leaving residual concentrations rich in iron, aluminium, nickel and so on, depending on the composition of the rocks, so that the location of old oceanic or mantle rocks is of major consideration. Although chemical erosion is most active in humid, hot areas, palaeoclimatic considerations are generally not important as residual deposits formed in ancient tropical latitudes are readily eroded mechanically unless they are cemented, and, if cemented, are unlikely to be economic. Chemically inert minerals, containing gold, silver, tin, tungsten, and so on, may be removed in streams and deposited later as placers. The placer deposits tend to be richer nearer to the primary ore source so that they often form marginal to present day or old subduction and collision zones. More soluble minerals, such as the copper porphyries of a subduction zone, may also be carried short distances in arid climates, forming red bed coppers, such as those of the intermontane basins of the Andes[28], the Colorado Plateau[40] and the Fergana Basin (USSR), but such deposits, often containing uranium, vanadium and other heavy mineral concentrations, are susceptible to later chemical erosion and only deposits formed during the past 200 m.y. are likely to be still present in any quantity, unless preserved by impermeable rocks. Most metal sulphides, originally formed within subduction zones, are likely to be dissolved and carried away, mainly as chloride complexes and organogenic compounds, particularly if saline waters are available[41-46]. This chemical removal of metallic salts from active or recently active subduction zones would normally be effectively complete in a short time, possibly some 10–20 m.y., and most solutions would be carried directly into the sea to become disseminated throughout the oceans. But some salts may enter environments where precipitation occurs and, under very specialized conditions, economically significant concentrations may form[8].

Anaerobic conditions, especially with organic sulphur, seem to be a favourable geochemical environment on the continental block in which copper, lead and zinc sulphides are precipitated[47-49]. Such an environment could occur under a variety of circumstances, so it is necessary to consider the factors which optimize the conditions under which significant quantities of metal sulphides may be precipitated. Unfortunately such an examination must be based on the characteristics of known reserves as most geochemical factors are complex and still inadequately known. Obviously the ground waters would be richest nearest to the original metal concentration, such as a subduction zone, and the content would tend to be highest where the highest salinities, temperatures and organic-chemical activity, that is, in low-latitude, arid areas[50], often closely associated with areas of very high organic content. The copper sulphides of the European Kupferscheifer beds, the Katangan-Zambian Copperbelt, the White Pine (Michigan) and Kazakhstan areas appear to have deposited in these low latitude conditions.

The origin of the Mississippi Valley type lead and zinc sulphides, in areas such as Tri State (USA), and the European Silesian ores, has long been in dispute[47,51,52]. The absence or very small scale of obvious igneous activity or feeders and the sedimentary or early replacement nature of many of the stratiform ores indicate that they have not formed directly by igneous activity. The carbon, oxygen[53] and sulphur[46,47,54] isotopes are of shallow origin and have been initially incorporated in the minerals at about the same time or shortly after the deposition of the host limestones. Saline inclusions within the ores, however, indicate crystallization temperatures around 100° C, but ranging between 50 and 200° C (ref. 56), and this has been interpreted to mean that igneous heating is necessary to warm metal bearing brines, even if igneous rocks are not necessary as a source for the metals. But ground waters rich in metals react with sulphur in organic-rich pockets and precipitate lead and zinc sulphides and the exothermic reactions within the organic material, especially between hydrocarbons and anhydrite[55,57], could cause localized high temperatures and the deposition of the sulphides itself appears to be strongly exothermic. Such temperature rises need be only small in low latitudes as the temperature of ground waters near the surface already falls within the lower temperature range.

Hydrocarbon-anhydrite reactions would also be associated with the low latitude[50] "sabkha" evaporite formations[58]. Such an explanation for the genesis of these ores has several other implications because they should be approximately contemporaneous (within some 10 m.y.) with the subduction zones from which they originated and would be restricted to a comparatively short time interval when such rich ground waters were available. The ores would also be approximately contemporaneous with the limestones in which they are found because the organic reactions would be maximized shortly after the accumulation of the organic material, and the ores would therefore decrease with depth, reflecting the change in the original organic quantity and activity. The movement of ground water through the chiefly impermeable basement rocks would be strongly influenced by any fracture patterns and basement topography and such patterns would also influence the formation of solution cavities in the overlying limestones, which could fill with organic material in near-shore, shallow marine anaerobic conditions. Both factors would tend to concentrate lead and zinc deposits over basement "high" areas, particularly over intersecting fractures in the underlying basement[59,60].

Any of these ores may have been subjected to later metamorphism, structural dislocation[61] and mobilization as later subduction zones or continental collisions occurred[62], as in the Swedish and Australian major lead and zinc deposits and the copper ores of the African Copperbelt. Nonetheless, even in such complex areas, a knowledge of the conditions of initial formation and therefore the probable source rocks is important in planning future development, and the location of such ores in less complex situations can be assessed more readily from a knowledge of the location and age of past subduction and collision zones and of the palaeoclimatic conditions bordering these areas while they were being eroded.

Significance for Mining

The application of plate tectonic concepts within ore genesis has a variety of consequences. The principal feature is that ores associated with igneous activity must be concentrated in specific areas at specific times and in a determinable order and age sequence. Such concepts are readily applicable to present subduction areas and allow determination of potential ore reserves from fairly simple considerations. For many other ore deposits the location of past subduction zones and slices of oceanic/mantle exposures are of critical importance but the most surprising consequence is that it affords an understanding of the conditions in which major sedimentary ores may have formed. This has been problematical for many decades and is important both for locating new reserves and exploiting known ore bodies. Such considerations need very much closer examination, particularly in testing the apparent low-latitude control of the deposition of such major ore bodies, and it is not to be expected that all ore bodies will fit into any simple mechanism[63]. Nonetheless, the changing position of the continents in respect to subduction, collisions and palaeoclimate will clearly be of much greater importance in the future. As known resources become exhausted and technology changes, the economic factors change, so that poorer grades of ore will become workable. But exploration costs for the richer ores will also increase and it will therefore become essential to localize areas in which the more sophisticated and increasingly expensive exploration tools are applied. Perhaps more fundamentally, such considerations should allow a basis for evaluating the real potential and location of the Earth's remaining natural mineral resources.

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Natural Units in Atomic and Molecular Physics

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Professor McWeeny's account includes a list of natural units for the most common quantities in terms of four base units.

In theoretical atomic and molecular physics it is often convenient to use "atomic units". Such units have various advantages. In the first place they are "natural" units, provided by naturally occurring quantities such as the magnitude of the electronic charge (e) and the "rationalized" Planck constant ($h/2\pi$), and are therefore independent of the practical units in terms of which their values can be assigned only by experiment: the charge on the electron does not depend on either measurement or international agreement, but its value in terms of the Coulomb does. Second, their adoption permits all the fundamental equations to be written in a dimensionless form in which constants such as e, m and h are absent and need not be considered at all during mathematical derivations or the processes of numerical solution; the units in which any calculated quantity must appear are implicit in its physical dimensions and may be supplied at the end. Although atomic units have been very widely used since the introduction by Hartree of the "double-Rydberg", and although the "hartree" and the "bohr"[1] have been quite widely adopted as units of energy and length, no complete discussion of natural units seems to be available in the literature. With the advent of the Système International (SI) d'Unités, the proposals of Shull and Hall[1] seem to merit reappraisal and extension.

In this article I shall give a fairly complete list of natural units for all the most commonly encountered quantities in terms of four base units, and I shall propose names for some of the units; the values of all of them are given here in terms of practical SI units. I shall also give two examples to illustrate the use of the units in reducing complicated equations to a simple dimensionless form.

Units

In the quantum mechanical treatment of the hydrogen atom (fixed nucleus approximation) there occur the quantities

$$a_0 = \hbar^2 \kappa_0 / me^2, \quad E_0 = me^4/\kappa_0{}^2\hbar^2 = e^2/\kappa_0 a_0 \qquad (1)$$

where a_0 is the radius of the first Bohr orbit and E_0 is the magnitude of the electronic energy in the ground state. The abbreviations used are $\hbar = h/2\pi$ (the rationalized Planck constant) and $\kappa_0 = 4\pi\varepsilon_0$ where ε_0 is the permittivity of free space. These quantities are the SI equivalents of those for which Shull and Hall[1] proposed the names bohr and hartree

$$a_0 = 1 \text{ bohr (B)}, \qquad E_0 = 1 \text{ hartree (H)}$$

The most convenient base units seem to be the mass of the electron (m), the magnitude of the electronic charge (e), the Planck constant ($\hbar$), and the permittivity (κ_0); in terms of these, the bohr and the hartree are "derived units". The natural units of all other physical quantities are easily expressed as dimensionally appropriate products of the form $m^p e^q \hbar^r \kappa_0{}^s$ where only integral powers occur.

Table 1 Base Units

Quantity and dimensions *	Natural unit and name †	SI equivalent ‡
Mass M	$m = 1$ em (electron-mass)	9.1091×10^{-31} kg
Charge Q	$e = 1$ e (electron)	1.60210×10^{-19} C
Action MLT^{-1} (A)	$\hbar = 1$ P (planck)	1.05450×10^{-34} J s
Permittivity $Q^2W^{-1}L^{-1}$	$\kappa_0 = 4\pi\varepsilon_0$ (no name)	$4\pi \times 8.8542 \times 10^{-12}$ F m^{-1}

* The dimensions of all quantities are expressed in terms of the more conventional symbols M, L, T, Q. For convenience, A (action) and W (energy) are also frequently employed.

† The choice of m, e, $\hbar$ and κ_0 as base units fixes all others. There is no generally accepted set of names for the natural units.

‡ The SI base units required in these tables are the metre, kilogram, second and ampere (m, kg, s, A). The other important named units are the joule (J), newton (N), coulomb (C), watt (W), volt (V), farad (F), weber (Wb), henry (Hn) and tesla (T). (The henry is here denoted by Hn to avoid confusion with the Hartree atomic unit, H). For further information see ref. 3.

The base units of mass, charge, action and permittivity, and their values in SI units are indicated in Table 1, together with suggested names. The "planck" is obviously appropriate as the name of the unit of action; and the "electron mass" (or "em") and the "electron" (or "e") seem to be uncontroversial. The derived units are listed in three groups in Tables 2, 3 and 4, together with their dimensions in mass, length, time and charge (M, L, T, Q) and their SI values.

Table 2 Derived Units (Mechanical)

Quantity and dimensions	Natural unit and name*	SI equivalent†
Length L	$\kappa_0\hbar^2/me^2=1$ B (bohr, a_0)	5.29167×10^{-11} m
Time T	$\kappa_0{}^2\hbar^3/me^4=\hbar/E_0=1$ as ("atomic second")	2.41889×10^{-17} s
Velocity‡ LT^{-1}	$e^2/\kappa_0\hbar=a_0E_0/\hbar=1$ B as^{-1}	2.18764×10^{6} m s^{-1}
Force MLT^{-2}	$m^2e^6/\kappa_0{}^3\hbar^4=E_0/a_0=1$ H B^{-1}	8.23831×10^{-8} N (J m^{-1})
Energy ML^2T^{-2} (W)	$me^4/\kappa_0{}^2\hbar^2=e^2/\kappa_0a_0=1$ H (hartree, E_0)	4.35944×10^{18} J
Power ML^2T^{-3}	$m^2e^8/\kappa_0{}^4\hbar^5=E_0{}^2/\hbar=1$ H as^{-1}	1.80225×10^{-1} W (J s^{-1})

* The names bohr and hartree are fairly widely used for the units of length and energy. For the atomic unit of time the name "atomic second" is proposed, with the symbol "as" formed by adding the prefix "a" to the conventional symbol "s". Each unit is expressed first in terms of the primary units (m, e, $\hbar$, κ_0) of Table 1 and, wherever it is more convenient, in terms of a dimensionally equivalent combination of derived units.

† The expressions in parentheses indicate dimensionally equivalent units (for example, 1 N=1 J m^{-1}) corresponding to those employed in column 2.

‡ In these units the velocity of light is 137.030 B as^{-1}, the fine structure constant being $\alpha=e^2/\kappa_0\hbar c=1/137.030$.

Table 3 Derived Units (Electrical)

Quantity and dimensions	Natural unit and name*	SI equivalent†
Electric permittivity $Q^2W^{-1}L^{-1}$	$\kappa_0(=4\pi\varepsilon_0)=1$ e^2 H^{-1} B^{-1}	$4\pi\times8.8542\times10^{-12}$ F m^{-1} (C^{2}J^{-1} m^{-1})
Electric current QT^{-1}	$me^5/\kappa_0{}^2\hbar^3=eE_0/\hbar=1$ e as^{-1}	6.62329×10^{-3} A
Electric potential WQ^{-1}	$me^3/\kappa_0{}^2\hbar^2=E_0/e=1$ aV ("atomic volt")	2.72108×10^{1} V
Capacitance Q^2W^{-1}	$\kappa_0{}^2\hbar^2/me^2=e^2/E_0=1$ e^2 H^{-1}	5.88774×10^{-21} F (C^2 J^{-1})
Electric field strength (E) $WQ^{-1}L^{-1}$	$m^2e^5/\kappa_0{}^3\hbar^4=E_0/ea_0=1$ aV B^{-1}	5.14220×10^{11} V m^{-1}
Electric displacement (D) QL^{-2}	$m^2e^5/\kappa_0{}^2\hbar^4=e/a_0{}^2=1$ e B^{-2}	5.72142×10^{1} C m^{-2}
Electric dipole moment LQ	$\kappa_0\hbar^2/me=ea_0=1$ e B	8.47778×10^{-30} C m
Electric polarizability $L^2Q^2W^{-1}$	$\kappa_0{}^4\hbar^6/m^3e^6=e^2a_0{}^2/E_0=1$ e B^2 aV^{-1}	1.64867×10^{-41} F m^2

* Each unit is expressed first in terms of the base units (m, e, $\hbar$, κ_0) of Table 1 and, wherever it is more convenient, in terms of a dimensionally equivalent combination of derived units. The "atomic volt" (aV) is proposed as a convenient unit of potential.

† The expressions in parentheses indicate dimensionally equivalent units (for example, 1 N=1 J m^{-1}) corresponding to those employed in column 2.

The "bohr" and the "hartree" appear in Table 2 and are widely accepted already; the "atomic second" (1 as) has the dimensions of action/energy and could thus be written as 1 P H^{-1} but it seems desirable to introduce a corresponding named unit and the addition of a prefix "a" to the symbol for the practical unit appears to be convenient and unobjectionable. As the fine structure constant $\alpha=e^2/\kappa_0\hbar c=1/137.030$ it follows that a photon travels 137.030 B in 1 as.

In Table 3, the "atomic volt" (aV) has been introduced as the natural unit of potential; 1 hartree (27.2108 eV) of work is performed in moving 1 e between points differing in potential by 1 aV and hence 1 aV=27.2108 V. The only other new name introduced (Table 4) is the "atomic tesla" (aT), for the natural unit of magnetic flux density. The significance of the unit may be visualized by reference to the Biot–Savart law: for unit charge (e), moving in a circular orbit, the flux density at the centre would be

$$\mathbf{B}=(\mathbf{r}\times e\mathbf{v})/((\kappa_0c^2)r^3)=e\mathbf{L}/m(\kappa_0c^2)r^3$$

For unit angular momentum (**L**) and unit radius (**r**) this would give a flux density $(137.030)^{-2}$ aT. The atomic tesla corresponds to an extremely strong field ($\sim2\times10^5$ T), but fields in atoms may evidently be of the order 10^{-4} aT, or 20 T (that is 20 kgauss). The unit is convenient in discussing spin interactions: the Bohr magneton ($\beta=e\hbar/2m$) represents half an atomic unit of magnetic moment, and a field of flux density 1 aT would be required to produce a free-electron ($g=2$) Zeeman splitting of 1 H.

Dimensionless Equations

In equations relating physical quantities it is customary to use the conventions of "quantity calculus" in which the symbols occurring stand for the quantities themselves: in this case dimensional consistency must be preserved in all mathematical manipulations and a term such as $\log(p/p_0)$ is acceptable (logarithm of a number) whereas $\log p$ (logarithm of a quantity, pressure, for example) is unacceptable. An alternative convention is to interpret the symbols as the numerical measures of the quantities they represent, referred to some specified system of units: in this case the equations contain only pure numbers or dimensionless variables; the ordinary processes of mathematical analysis apply without further reference to units, and the appropriate units are supplied at the end of a calculation, by reference to the physical dimensions of the quantity calculated. The second convention has much to recommend it and is tacitly accepted in atomic and molecular physics whenever atomic units are introduced, for example for convenience in computation. Here I simply give two examples to illustrate its advantages.

Electric Polarizability of the H Atom

With a field E along the z axis the unperturbed Hamiltonian operator ($H^{(0)}$) and the perturbation ($H^{(1)}$) are given by

$$H^{(0)}=-(\hbar^2/2m)\nabla^2-e^2/\kappa_0r,\quad H^{(1)}=Eez$$

As any dimensionally consistent equation may be replaced by

Table 4 Derived Units (Magnetic)

Quantity and dimensions	Natural unit and name*	SI equivalent†
Magnetic permeability MLQ^{-2}	$\kappa_0\hbar^2/e^4=ma_0/e^2=1$ em B e^{-2}	1.87797×10^{-3} N A^{-2}
Magnetic flux WTQ^{-1}	$\hbar/e=1$ P e$^{-1}=1$ aV as	6.58199×10^{-16} Wb (V s)
Magnetic flux density (B) $MT^{-1}Q^{-1}$ (magnetic induction)	$m^2e^3/\kappa_0{}^2\hbar^3=\hbar/ea_0{}^2=1$ aT ("atomic tesla")	2.35055×10^{5} T
Magnetic inductance WT^2Q^{-2}	$\kappa_0{}^2\hbar^4/me^6=\hbar^2/e^2E_0=1$ aV as^2 e^{-1}	9.93764×10^{-14} Hn (Wb A^{-1})
Magnetizing force (H) $QL^{-1}T^{-1}$	$m^2e^7/\kappa_0{}^3\hbar^5=E_0e/a_0\hbar=1$ H aT^{-1} B^{-3}	1.25165×10^{8} A m^{-1} (J T^{-1} m^{-3})
Magnetic dipole moment ‡ L^2QT^{-1}	$e\hbar/m=E_0ea_0{}^2/\hbar=1$ H aT^{-1}	1.85464×10^{-23} J T^{-1}
Magnetic susceptibility $L^2Q^2M^{-1}$ (induced moment/flux density)	$\kappa_0{}^2\hbar^4/m^3e^2=e^2a_0{}^2/m=1$ H aT^{-2}	7.89023×10^{-29} J T^{-2}

* Each unit is expressed first in terms of the base units (m, e, $\hbar$, κ_0) of Table 1 and, wherever it is more convenient, in terms of a dimensionally equivalent combination of derived units. The "atomic tesla" (aT) is proposed as a convenient unit of flux density.

† The expressions in parentheses indicate dimensionally equivalent units (for example, 1 N=1 J m^{-1}) corresponding to those employed in column 2.

‡ The Bohr magneton ($\beta=e\hbar/2m$) corresponds to $\frac{1}{2}$ atomic unit of magnetic moment: the Zeeman splitting for a free electron ($g=2$) in a field of flux density 1 aT would be 1 H.

one in which all quantities have been replaced by their numerical measures, the corresponding dimensionless operators are (replacing $\hbar$, e, m, κ_0 by 1 throughout)

$$H^{(0)}=-\tfrac{1}{2}\nabla^2-(1/r),\quad H^{(1)}=Ez \tag{2}$$

All symbols now refer to natural units (for example, the distance of the electron from the nucleus is r bohrs or ra_0). The unperturbed Schrödinger equation $H^{(0)}\psi=\varepsilon\psi$ has a normalized ground state solution

$$\varepsilon^{(0)}=-\tfrac{1}{2};\ \psi^{(0)}=(1/\sqrt{\pi})\exp(-r) \tag{3}$$

The first order perturbation $\psi^{(1)}$ satisfies the equation

$$(H^{(0)}-\varepsilon^{(0)})\psi^{(1)}=-(H^{(1)}-\varepsilon^{(1)})\psi^{(0)}$$

which becomes, in natural units,

$$(-\tfrac{1}{2}\nabla^2-1/r+\tfrac{1}{2})\psi^{(1)}=-Ez\psi^{(0)}$$

The solution is readily obtained in the form

$$\psi^{(1)}=-z(1+\tfrac{1}{2}r)\psi^{(0)} \tag{4}$$

and the second order perturbation energy follows as

$$\varepsilon^{(2)}=\langle\psi^{(0)}|H^{(1)}|\psi^{(1)}\rangle=-(9/4)E^2 \tag{5}$$

The electric polarizability α is defined by writing this result as $-\frac{1}{2}\alpha E^2$ and is thus $\alpha=9/2$. The natural units of all quantities may be supplied from the tables, or from first principles. Thus polarizability is electric moment/field, where electric moment has the unit 1 e B and field the unit 1 aV= 1 H e^{-1} B^{-1} (field=potential gradient=energy × charge^{-1}/distance), and hence α has the natural unit 1 e B aV^{-1}=1 e^2 B^2 H^{-1}. In absolute units therefore (see Table 2 or equation (1))

$$\alpha=4.5\ e^2a^2{}_0/E_0=4.5\ \kappa_0{}^4\hbar^6/m^3e^6=4.5\times1.64857\ 10^{-43}\ \text{F m}^2$$

Any dimensionally equivalent statement may of course be used; for example (from equation (1)) $\alpha=4.5\ \kappa_0a_0{}^3$.

Similar considerations apply to the wavefunction: the dimensionless r is distance/a_0 and thus when r is reinterpreted as an actual distance the dimensionless r must be replaced by r/a_0. Also $|\psi|^2$ is a probability density and has dimensions L^{-3}: the normalizing factor in conventional units thus has dimensions $L^{-3/2}$ and the dimensionless factor must be multiplied by $a_0{}^{-3/2}$. Thus

$$\psi(r)=(\pi a_0^3)^{-1/2}\exp(-r/a_0) \tag{7}$$

It is seldom necessary to revert to conventional units, but this can always be done, even in the most complicated cases, by use of the tables.

Terms in the Dirac-Pauli Hamiltonian

After reduction to Pauli form (see, for example, McWeeny and Sutcliffe[2]) the Dirac equation for one electron in the presence of an electromagnetic field (**E** and **B** arising from scalar and vector potentials φ and **A**) may be written

$$H=\tfrac{1}{2}\boldsymbol{\pi}^2-e\varphi+\tfrac{1}{8}g\mathbf{B}\cdot\mathbf{S}+g\alpha^2(\mathbf{S}\cdot\mathbf{E}\times\boldsymbol{\pi}-\mathbf{S}\cdot\boldsymbol{\pi}\times\mathbf{E})$$

Here natural units have been employed throughout, so all symbols may be interpreted as the dimensionless measures of the quantities they represent; the operator $\boldsymbol{\pi}=\mathbf{p}+\mathbf{A}$ (where **p** is the usual momentum operator), α is the fine structure constant, and in Dirac theory $g=2$. Relativistic variation of mass has been neglected and an unimportant term in div **E** has been discarded as usual.

Suppose, having derived such an equation, or any results that follow from it, one wishes to supply the missing combinations of fundamental constants. When the symbols in equation (7) are reinterpreted as quantities, instead of dimensionless variables (that is their numerical measures in natural units), every term must take the overall dimensions of energy ($W=ML^2T^{-2}$). Thus $[\boldsymbol{\pi}^2]=[\text{momentum}^2]=M^2L^2T^{-2}=MW$ and the missing factor before the $\boldsymbol{\pi}^2$, whose dimensions are M^{-1} and whose measure in natural units is 1, must therefore be m^{-1}. Similarly, from Table 4, $[\mathbf{A}]=[\mathbf{B}]L=MLT^{-1}Q^{-1}$ and the missing factor before the **A** has dimensions Q. Thus $\mathbf{A}\rightarrow e\mathbf{A}$.

In the same way, if we use the common convention of retaining dimensionless angular momentum operators (**S**), the **B·S** term has dimensions $MT^{-1}Q^{-1}$ and therefore requires a factor with dimensions $WM^{-1}TQ$. As action (A)=energy × time, this is equivalent to $AM^{-1}Q$ and the missing factor is $\hbar e/m$.

Finally, as **E** has dimensions $WQ^{-1}L^{-1}=M^2LT^{-2}Q^{-1}$, it follows that $[\mathbf{S}\cdot\mathbf{E}\times\boldsymbol{\pi}]=M^2L^2T^{-3}Q^{-1}$ and the dimensions of the missing factor must be $WM^{-2}L^{-2}T^3Q=M^{-1}TQ$. Because the natural unit of time is $\kappa_0{}^2\hbar^3/me^4$ the factor must be $\kappa_0{}^2\hbar^3/m^2e^3$.

On putting these results into equation (7) and remembering that $\alpha=e^2/\kappa_0\hbar c$, one obtains the Hamiltonian

$$H=(\mathbf{p}+e\mathbf{A})^2/2m-e\varphi+g\beta\mathbf{B}\cdot\mathbf{S}+(ge\hbar/8m^2c^2)(\mathbf{S}\cdot\mathbf{E}\times\boldsymbol{\pi}-\mathbf{S}\cdot\boldsymbol{\pi}\times\mathbf{E}) \tag{8}$$

where $\beta=e\hbar/2m$ is the Bohr magneton and all terms are compatible with the use of SI units.

These examples are typical. Provided that SI conventions are respected, mathematical derivations and calculations may be performed entirely in terms of dimensionless variables, simply by discarding the constants e, m, $\hbar$ and $4\pi\varepsilon_0$, which become the natural units of charge, mass, action and permittivity. The results are immediately expressible in practical (SI) units by reference to the tables given. The mixed gaussian system does not possess sufficient internal consistency to be used in a similar way, the use of two definitions of charge leading to ambiguities and the appearance of spurious powers of c.

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New Evidence for a Relationship between Tobacco Smoking, Nicotine Dependence and Stress

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Rats which have learned to avoid an electric shock under the influence of nicotine can become dependent on nicotine for a successful performance. Evidence is presented which suggests that this dependence is related to the degree of stress to which the animal is exposed.

It is now reasonable to assume that many inhaling cigarette smokers become dependent on the nicotine content of tobacco smoke, although why this dependence develops is not yet fully understood. Studies in experimental animals point primarily to nicotine and cigarette smoking having a stimulant action[1,2], and this could explain the subjective impression that cigarette smoking in man improves concentration and efficiency[1]. The demonstration by Frankenhaeuser *et al.*[3,4], that cigarette smoking facilitates the performance and enhances the efficiency of habitual smokers in a behavioural situation characterized by monotony, would appear to support such a hypothesis. This seems unlikely to be the complete explanation for the development of the smoking habit, however, as dependence on nicotine probably results from exposure to a variety of emotional circumstances. On the one hand, the arousal effects of nicotine could improve concentration and therefore the overall capabilities of the smoker, enabling him to cope more satisfactorily with an allotted task without feeling anxious. This may explain the subjective impression of some smokers that they are calmed by smoking a cigarette. On the other hand, studies of the environmental circumstances which may contribute to the development of the smoking habit suggest that nicotine, in the medium of tobacco smoke, may help to alleviate the effects of stress mediated by psychological or physical discomfort. Smokers do appear to smoke, or to smoke more, when exposed to an emotionally stressful situation[5]; this could apply to 70% of heavy smokers and 30% of light smokers[6]. From the available evidence it seems that the experience of stressful situations can contribute not only to the beginning of the smoking habit, but also to its continuation and to the number of cigarettes consumed. We believe that the experiments reported here provide new evidence, albeit from animal studies, for a relationship between cigarette smoking, nicotine dependence and alleviation of stress.

Experiments were carried out with rats which were trained to avoid electric shocks by pressing a lever[7]. Rats have difficulty in learning this type of behaviour[8], and the stressful nature of avoidance experiments has previously been demonstrated[9]. By modifying the experimental situation we showed that the effects of nicotine can be related to the degree of stress to which the animal is exposed.

Nicotine in a Stressful Situation

Male and female Sprague-Dawley and cross-bred (Sprague-Dawley/Lister black-hooded) rats were trained to press a lever on a shock avoidance schedule. Sixteen pairs of rats of the same sex, strain and age were used, one animal receiving 0.4 mg nicotine kg^{-1} subcutaneously immediately before each training session (nicotine-trained), the other receiving saline (saline-trained). The dose of nicotine, as the hydrogen tartrate, is expressed in terms of the base. During training, electric shocks lasting 0.5 s were delivered through the grid floor of the chamber every 3 s. Each lever-pressing response restarted the timer and so postponed the next shock for 30 s. Shock levels were increased during training from 0.1 to 0.4 mA. Some rats took longer to train than others. But when the rats had learned to avoid most of the shocks they were placed on a final schedule in which 0.4 mA, 0.5 s shocks were programmed every 30 s, each lever-pressing response postponing the next shock for 30 s. The first day on this final schedule was considered as the first day of the experiment. Each rat was run singly for 60 min daily at the same time for 5 d a week throughout both the training period and the experiment.

Saline was substituted for nicotine on the fourth or twelfth day of the experiment and this substitution was repeated at intervals of 1 week on three other occasions. Nicotine was given before the session on all other days. The saline-trained rats received saline before each session throughout the experiment.

At the beginning of the experiment the nicotine-trained rats pressed the lever more often and received fewer shocks than their saline-trained partners. During the first 3 d of the experiment thirteen out of sixteen nicotine-trained rats took fewer shocks than their partners ($P<0.05$, Wilcoxon matched-pairs, signed-ranks test[10]). When saline was substituted for nicotine the number of shocks received by the nicotine-trained rats increased to such an extent that most took more shocks than did their saline-trained partners on the same day. The difference was not quite significant for the first two occasions on which nicotine was replaced by saline, but significantly more shocks were taken by the nicotine-trained rats on the third and fourth occasions ($P<0.01$, Wilcoxon matched-pairs, signed-ranks test). It thus seemed that the nicotine-trained rats had become dependent on nicotine for their continued successful performance and that in its absence they coped less effectively with the situation than did rats which had never experienced the drug.

Fig. 1 shows the number of shocks received by two of the pairs of rats studied. In the first pair, there was a large and persistent disruption of behaviour when saline was substituted for nicotine, with an approximately four-fold increase in the number of shocks received. In the second pair, the nicotine-trained rat took progressively more shocks after repeated saline substitutions.

The simplest explanation for the poor performance of the saline-substituted rats is that they were unable to remember the response in the absence of nicotine. This has been referred to as dissociation of learning[11]. Fig. 1, however, shows that it was possible for a rat to perform adequately during the first saline substitution—indicating that the behaviour was remembered—but then to give a progressively poorer performance after subsequent substitutions. Two pairs of nicotine-trained rats, in which saline was repeatedly

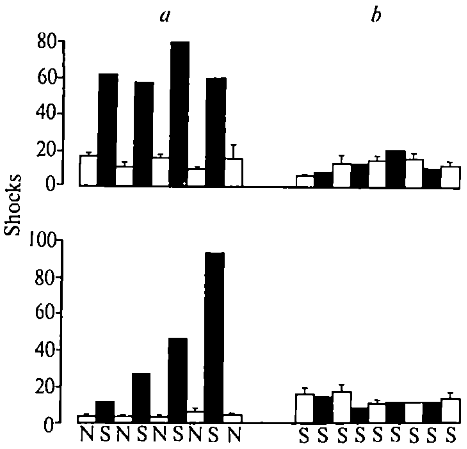

Fig. 1 Number of shocks received by two pairs of rats. *a*, Nicotine-trained; *b*, saline-trained. Solid columns, no. of shocks on four occasions at 1 week intervals on which saline was substituted for nicotine; open columns, mean shocks on the intervening days and on the 3 d preceding the first and following the last substitution with standard errors. S, Saline, N, 0.4 mg nicotine kg^{-1} before the sessions.

substituted for nicotine, still showed disruption of behaviour on the thirteenth occasion on which nicotine was withdrawn. This provides further evidence that disruption of behaviour was not caused by dissociation of learning, as these animals had the opportunity during this time to relearn the avoidance response in the presence of saline.

The disruption of behaviour which occurred after substitution of saline for nicotine was not confined to any particular strain or sex. The severity of the disruption did not seem to depend on the time at which saline was first substituted for nicotine, as withdrawal of nicotine on the fourth or the twelfth day of the experiment produced a comparable deterioration in performance.

A second series of experiments was performed to study the effects of withdrawing nicotine in a less stressful situation. In these experiments the avoidance schedule differed only in that a 10 s white-noise feedback stimulus was presented each time the lever was pressed. This "safety" signal reduces the stressful effects of an avoidance situation[9]. On the two occasions on which nicotine was withdrawn in these conditions the nicotine-trained and saline-trained rats did not differ in the number of shocks received. When the "safety" signal was discontinued, however, and saline substituted for nicotine on two further occasions, the performance of the nicotine-trained rats deteriorated.

From these experiments, it seems that rats which have learned, while under the influence of nicotine, to avoid an electric shock in the absence of any feedback signal, can become dependent on nicotine for an effective performance. The presence of a "safety" signal lessens this dependence, possibly by reducing the degree of stress to which the animal is exposed.

The stressful effects of unsignalled avoidance schedules have previously been demonstrated in rats and monkeys[9,12]. Intensive training on an unsignalled avoidance schedule produced marked gastric ulceration in rats[9], although little ulceration developed in animals which were given a brief feedback or "safety" signal after each avoidance response. In rhesus monkeys, studies of the neuroendocrine response to stress showed that although restraint, or lever-pressing for food, did not influence plasma 17-hydroxycorticosteroid levels, a four-fold increase in plasma corticosteroids occurred when the animals were placed on an unsignalled avoidance schedule[12]. In contrast, when the avoidance procedure included an exteroceptive warning stimulus signalling the imminence of shock, the corticosteroid response was considerably reduced. When a "safety" signal was used in the experiments now reported, the degree of stress to which the rats were exposed was likely to be considerably reduced, and this probably accounts for the somewhat minimal disruption of behaviour when saline was substituted for nicotine in these conditions. This evidence may serve to relate the degree of dependence on nicotine to a particular level of stress.

The pharmacological effects of cigarette smoking can be mimicked by a series of small intravenous injections of nicotine[1,2]. After the first series of experiments, intravenous cannulae were inserted into the jugular vein of four of the nicotine-trained rats, the usual aseptic precautions being observed throughout the operative procedures. A "smoking dose" of nicotine (2 or 4 μg kg^{-1}) administered intravenously every 30 s for the 30 min preceding an experimental session substituted effectively for the usual subcutaneous dose of nicotine (Fig. 2).

Nicotine and Stress Mechanisms

Administration of nicotine to various species causes an appreciable and consistent increase in plasma[13] and adrenal (unpublished results of D. J. K. Balfour) corticosteroid levels. It has been suggested that effective behaviour depends on some optimum level of stress[14] and the development of successful avoidance behaviour in the rat has been shown to be accompanied by increased plasma corticosteroid levels[15]. Thus, if a facilitated adrenocortical response is necessary for effective behaviour, this might explain the development of dependence on nicotine after repeated exposure to an emotionally stressful situation. But recent studies have indicated that the initial increase in plasma corticosteroid levels which follows the intravenous administration of nicotine is not maintained even if the injections of nicotine are continued (unpublished results of D. M. Turner).

An alternative explanation for the mechanism through which nicotine alleviates the effects of stress is suggested by the results of recent experiments. Nicotine injected intravenously, or perfused through the third cerebral ventricle of the cat brain, causes an increase in the release of noradrenaline from the hypothalamus[16]. These observations are of particular interest, as the release of corticosteroids from the adrenal cortex in response to stress can be inhibited by infusing noradrenaline directly into the third cerebral ventricle[17]. In stressful situations, nicotine might therefore act by releasing noradrenaline from the hypothalamus, resulting in the inhibition of corticosteroid release from the adrenal cortex. To test this hypothesis we have carried out a preliminary series of experiments in the anaesthetized cat. We have shown that nicotine perfused through the hypothalamic region of the third cerebral ventricle can cause a reduction in circulating plasma corticosteroids. This effect is only observed if resting corticosteroid levels are relatively high as a result of surgical stress and exposure to

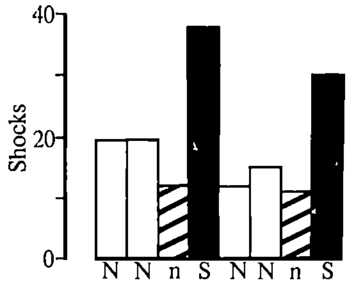

Fig. 2 Effect of intravenous nicotine on the number of shocks received by a nicotine-trained rat. Each column represents an experimental session. N, 5 μl saline intravenously every 30 s for 30 min followed by 0.4 mg nicotine kg^{-1} subcutaneously; n, 2 μg nicotine kg^{-1} intravenously in 5 μl saline every 30 s for 30 min followed by saline subcutaneously. S, Saline intravenously and subcutaneously.

anaesthetic. These preliminary findings offer additional evidence for a relationship between nicotine dependence and the alleviation of stress.

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Competition between Nerves for Functional Connexions with Axolotl Muscles

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This article provides a reinterpretation of the phenomenon of coordinated movement of grafted salamander limbs and suggests a mechanism for modifiable connexions between excitable cells.

SALAMANDER limbs implanted near those of a host become innervated and begin to move in time with movements of the limbs of the host animal. This ability was ascribed by Weiss to a message that ascended motoneurone axons and was able to readjust the impulse firing pattern of the motor nerve cells. Each motoneurone that grew a fibre into the grafted limb would have to change in some way so as to select from the central nervous system those excitations that were appropriate for the muscle in which the axon now terminated, a process which Weiss suggested could be called myotypic modulation. The other hypothesis to account for functional recovery of limb coordination, that of selective reconnexion of nerves with their own kind of muscle, was discarded by Weiss when he found that the pattern of innervation of the graft was not the same as that of the normal limb either in segmental origin or distribution to the muscles[1]. Myotypic modulation or respecification of motoneuronal function was also proposed to account for the good recovery of coordination of the salamander's own limbs when the nerve supply was cut and allowed to regenerate in an apparently haphazard manner[2].

This is not, however, the mechanism for the excellent recovery of fish fin or eye muscle coordination after similar reinnervation experiments[3,4]. Myotypic modulation has not been found, no readjustment of impulse discharge of motoneurones has been detected and muscles do become functionally reinnervated by their own motoneurones[5–9].

We now have evidence that much the same thing happens in reinnervation of salamander limbs. Even after maximum disorganization of the nerve supply to the whole limb a high degree of order, replicating the original pattern of nerve–muscle connexion, is apparent in the end result of functional reinnervation.

The two principal nerve trunks supplying one hindlimb of axolotls (larval forms of *Ambystoma mexicanum*) were broken half way down the limb, pulled back central to the pelvic girdle, crossed and pushed back down the wrong side of the ischium. The gross misdirection of nerve trunks at the root of the limb was maintained for at least 9 months. Nerves regenerated from the central cut ends of the trunks, each one in the wrong part of the limb, and came to resupply the limb muscles. No attempt was made to aid regeneration by suturing or apposing nerves and no anatomical barrier at this level would guide or restrict the extent of nerve growth. The leg, which was initially completely paralysed, began to move in 8 weeks and had recovered by 3 months. All movements were in the normal direction. No sign of reversed movement was seen.

Animals were killed at intervals from 45 d to 9 months and the hind limbs set up for stimulating the peripheral cut ends of each of the spinal nerve roots and, after the first two experiments, for recording the evoked junctional potentials by intracellular micro-electrodes in the flexor musculature of the limbs. Up to 200 penetrations were made over the flexor surface of each leg and the response of each muscle fibre to stimulation of each of the spinal nerves that contributed to the limb was noted.

In this way a map of the distribution of functional segmental innervation of limb muscles could be made. All muscle fibres showed electrophysiological evidence of innervation by several nerve fibres but each segmental nerve territory in the musculature has a sharp border in that no muscle contraction was seen and no action potentials could be recorded from muscle fibres beyond it. The borders of some territories were contiguous and others overlapped. Normally the details of the pattern of segmental innervation of a pair of limbs in one animal are remarkably similar (Fig. 1). There are variations between animals as to which spinal nerves supply the limb and their exact muscle territories. For example, the eighteenth nerve sometimes contributes to limb muscle innervation as in Fig. 1 and sometimes it does not enter the leg at all.

After regeneration of crossed limb nerves the symmetrical pattern of segmental limb muscle innervation was restored (Fig. 2). In spite of being directed into the posterior aspect of the limb, motor fibres of the sixteenth nerve came to innervate anterior muscles, and the reverse was the case for the seven-

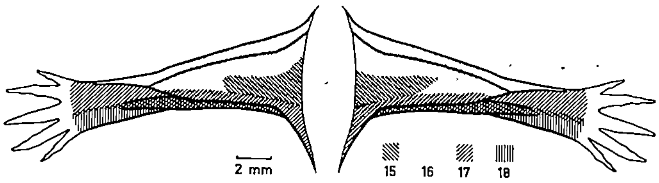

Fig. 1 Segmental innervation of muscles on the flexor surface of a pair of normal axolotl hind limbs. The map was prepared by stimulating the peripheral cut end of each of the spinal nerves 15–18, observing contraction and verifying areas of innervation by intracellular recording of evoked junctional potentials in muscle fibres. The limbs are seen from the ventral aspect with the anterior borders at the top. Note the symmetry of patterns of innervation. Scale is approximate.

teenth nerve. A small patch of muscle innervated by the fifteenth nerve was symmetrically disposed on each limb. This was the case in all animals (seven out of nine) allowed to survive long enough for good functional recovery, which we suggest could be largely a result of re-establishment of appropriate connexions between motor nerve fibres and muscles.

As each nerve trunk was initially directed into denervated muscle it is difficult to see how the growing nerve sprouts could avoid making their first contact with the wrong muscle fibres. Either they did not form neuromuscular junctions there or the ones they did form were trial synapses that did not remain functional. Provided nerve growth was profuse the eventual selection of the best set of nerve-muscle combinations could take place through interactions between a muscle cell and a number of convergent synaptic connexions more or less well-matched on embryological grounds, with the common post-synaptic muscle fibre. Competitive reinnervation of fish eye muscles by the original plus a foreign nerve results in the dominance of the correct innervation and a peripheral repression of the effectiveness of the competing innervation, without apparent degeneration or retraction of foreign nerve terminals[9].

There was no behavioural evidence of cross-innervation of limb muscles in the course of recovery, but this could have been concealed by stronger contractions of correctly connected muscles. We made microelectrode recordings only from 175 d after nerve section, by which time movement was fully restored. That a competitive mechanism is operative in reinnervation of axolotl muscles was discovered in the following way.

Cutting one of the spinal nerves which supplied a salamander limb leads to an expansion of the territories of muscle innervation of the adjacent segmental nerves[10,11]. We studied this process through two cycles of expansion, retraction when the severed nerve regrew into the limb and re-expansion when it was cut again.

Axolotls were prepared by cutting either the sixteenth or both the fifteenth and sixteenth spinal nerves on one side. Animals were allowed to survive for varying periods from 1 d to 6 months after which they were killed and the hind limbs mounted in a bath for electrical stimulation of the peripheral cut ends of the spinal nerves and microelectrode recording of junctional potentials in the flexor limb musculature. The pattern of innervation on the operated side, as shown by visual location of muscle contraction and confirmed by junctional potential recording, was compared with that of the opposite normal side. The limits of segmental territory were taken as areas in which maximal nerve stimulation at ten pulses per second caused no visible muscle contraction and no action potentials could be recorded by an intracellular microelectrode.

Immediately upon cutting a spinal nerve the dependent muscles were paralysed. Two or three days later the spread of muscle innervation first appeared as a marginal extension of the territories of adjacent nerve roots. The extension was only over about 0.5 mm but this was one-quarter to one-fifth of the width of the leg of the animals used and was clearly distinguishable from the pattern on the opposite leg. Muscle fibres in this area were innervated by terminals giving large junctional potentials with normal latency and strong muscle contraction. Between 1 and 2 weeks later the adjacent segmental innervation spread further and patches of innervated muscle fibres were detected in the central denervated zone. Junctional potentials tended to be smaller and the latency from nerve stimulation longer (Fig. 3). Thirty days after nerve section both kinds of foreign innervation began to disappear as the original innervation grew into the limb. Eventually the normal symmetrical pattern was re-established.

Cutting the sixteenth root a second time in animals that had recovered completely from a previous denervation resulted in an area of paralysed muscle fibres in the expected part of the limb. During the next 2 d there were no changes in the boundaries of territories of the other nerves, just as after the first denervation. In the limbs of animals killed from then on, however, the whole of the expected denervated area was cross-innervated by adjacent roots. After the first denervation this amount of spread took 3 weeks, not 3 d. Junctional potentials were of normal size and latency. There was no further change of innervation pattern in animals examined over the next 2 weeks (Fig. 4).

We suggest that the rapid spread of innervation occurring 3 d after cutting a nerve, either the first or second time, is a result of the emergency of function in pre-existing but functionless nerve terminals.

In normal limbs spread of innervation by this mechanism is confined to the margins of segmental territories. The later, patchy spread, appearing within 1 or 2 weeks and extending into the centre of a denervated area, could be a result of true collateral sprouting of remaining nerves (Fig. 5).

After allowing complete regeneration of the one original cut nerve back into the limb all foreign innervation, including that due to collateral sprouting, is repressed leading to the re-establishment of the normal pattern. When the same nerve is cut the second time, even 6 months later, foreign innervation is derepressed within 3 d, but as foreign terminals are now widespread in the limb the second spread of innervation is sudden and complete.

In normal axolotls we have examined the mode of termination of nerves extending into muscle beyond the zones of functional

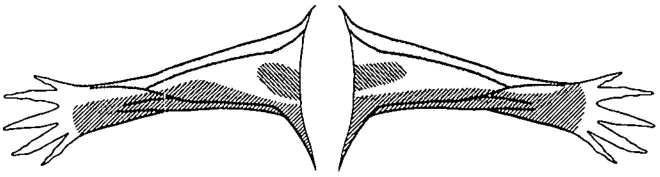

Fig. 2 Segmental innervation of muscles on the flexor surface of the hind limbs of an axolotl 9 months after breaking and crossing the major limb nerves. In this and subsequent figures the shading of nerve territories follows the convention of Fig. 1 and the experimental limb is on the left of the illustration, the untouched control limb on the right. In spite of disorganization of the nerve supply the functional innervation as revealed by intracellular junction potential recording on the operated side (left) mirrors that on the normal side (right).

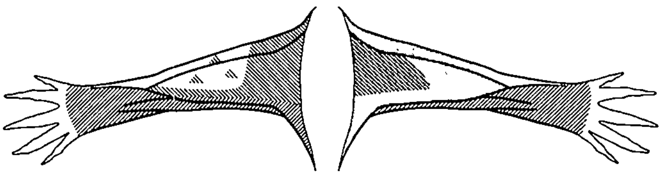

Fig. 3 Extension of the territories of muscle innervation on the flexor surface of an axolotl limb 7 d after cutting the sixteenth spinal nerve (left), as compared with the normal side (right). The territories of the fifteenth and seventeenth have expanded at the base of the limb. The triangles in the white area signify patches of muscle fibres innervated by the fifteenth nerve surrounded by non-innervated fibres.

innervation. Borders of spinal nerve territories were found by electrical stimulation and intracellular recording of junctional potentials in muscles. The corresponding nerve terminals were identified later using electron microscopy to detect neuromuscular junction made to degenerate partially by previous nerve section. Four days after cutting one spinal nerve muscle samples taken from either side of the previously defined functional border were found to contain a mixture of normal and degenerating synaptic terminals. Within the territory of the cut spinal nerve they were found in proportions of approximately three degenerating to one normal one; outside the territory there were three normals to one degenerating terminal. The normal synapses within the territory, and the degenerating one outside it, were presumably from the non-functional innervation, which therefore ends in neuromuscular junctions morphologically indistinguishable from normal synapses. This is also the case for repressed innervation of fish muscles[9].

The normal occurrence of mutual repression of competing synaptic connexions may explain how a sharp border of functional connectivity can be formed between segmental territories. For example, if the chemospecific nature of the muscle cells changes progressively from the anterior to posterior border of the limb, the sixteenth nerve will be favoured over the seventeenth anteriorly and the seventeenth over the sixteenth posteriorly. Competition towards the centre of a field, working by mutual repression of competing synaptic terminals, could result in an abrupt changeover in the dominance of connexions. Furthermore, 3 d, which is the time taken for dormant neuromuscular synapses to be reawakened, is barely long enough for detectable ultrastructural degeneration of nerve terminals after axon section. The first result of nerve section is to silence the innervation while that from adjacent roots remains active. Therefore, in the changeover zone, the relative amount of impulse traffic carried by mutually competing nerves, rather than their structural presence, may influence the functional connectivity that they establish with a common post-synaptic muscle cell. Experiments compatible with this interpretation have been reported briefly by Aguilar *et al.*[12], and others have been done by us. Therefore the expression of intercellular recognition mechanisms, on which the formation of ordered connexions are thought to depend, can apparently be modified by the relative impulse activity of competing synapses.

There is one other published account of apparently functionless cross-innervation in salamander limbs. Grimm crossed flexor and extensor muscle nerves in the forearm of axolotls and after recovery of limb movement tested connexions by electrical stimulation. The original nerve trunks, above the point of crossing, had regained control of the muscle they originally innervated, showing that the visible crossed nerves entering the muscles lower down were mainly ineffective and that contraction depended on a few fibres recrossing to get to their right muscles[13].

We suggest that the detailed pattern of muscle innervation in the development of these limbs depends upon widespread nerve growth and subsequent competitive interactions between nerve terminals for the control of muscles. Other developmental forces are apparently at work to direct nerve fibre growth to approximately the right place in the limb[14], so that normally the competition between nerves leaves only a fringe of functionless innervation on the borders between natural muscle territories. In a regeneration or transplantation experiment such as was originally carried out by Weiss, however, much of the anatomically traceable innervation is likely to be quite out of place in the limb and therefore functionally disconnected from muscles. It is easy to see now why he became convinced that the principles of specific anatomical connectivity could not explain the restoration of function in grafted supernumerary limbs.

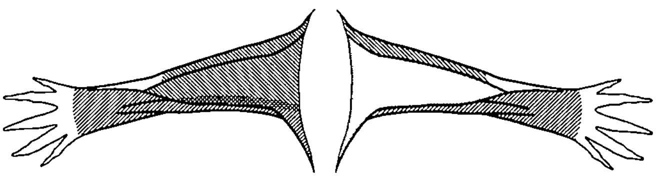

Fig. 4 Pattern of segmental innervation of flexor muscles of axolotl hind limbs 3 d after cutting the sixteenth root a second time. The animal had completely recovered from the first denervation done three months before. The whole of the territory of the sixteenth nerve on the operated side (left) is innervated by terminals from the fifteenth or seventeenth spinal nerves.

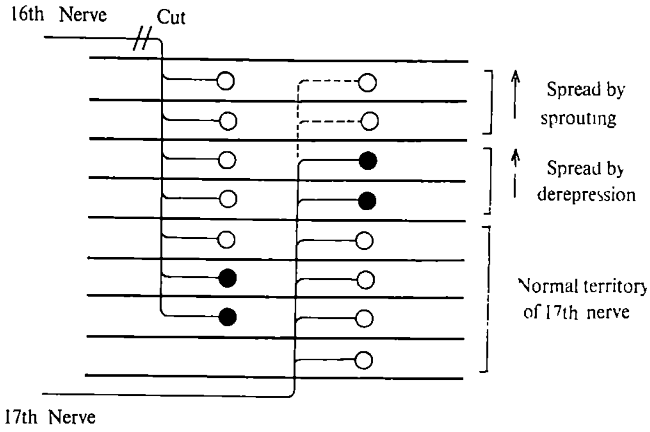

Fig. 5 Proposed mechanisms of spread of segmental innervation of muscle fibres. Repressed terminals (black) are normally present on the borders of spinal nerve territories and become functional upon silencing or degenerating out the competing innervation. This takes about 3 d. Further extension of innervation into denervated muscle is possible by sprouting of remaining nerves.

Both the mechanisms regulating functional nerve–muscle connexions could be important in the development and modifiability of interneuronal connexions in the central nervous system. Mutual repression of competing synapses may be required for the formation of topographically organized connexions between sets of interneurones such as are found in afferent pathways from the skin or eye. This may leave a fringe of functionless synapses just as in salamander muscle. Where the chemospecific preferences of cells are not so strong, as in densely interconnected neurones of higher levels of the nervous system the relative amount of use of a synapse may sway the recognition mechanisms in its favour, to the disadvantage of other convergent connexions. Use-dependent changes in connectivity during development, like those described in visual systems, could be attributable to a similar mechanism of mutual, functional repression of synaptic transmission, without immediate changes in ultrastructure[15].

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Cosmic Abundance of Boron

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The abundance of boron is of considerable interest, but is difficult to determine. Boron undergoes thermonuclear destruction in stellar interiors, and hence is not a normal product of stellar nucleosynthesis. Thus its abundance is low, and until now it has seemed possible to understand its production in nature as a consequence of cosmic ray bombardment of the interstellar medium[1]. We point out here that the cosmic abundance of boron has been greatly underestimated, and that the upward revision of its abundance has important consequences in several areas of astrophysics.

IN this article, we shall express all abundances relative to 10^6 atoms of Si. This requires conversion of abundances relative to 10^{12} atoms of H, using the factor 0.03175 (A. G. W. C., unpublished).

An average abundance of boron in ordinary chondrites[2,3] is 6.2. Boron is very difficult to measure quantitatively in meteorites, but recently it has become apparent that the boron abundance is highly variable, and in particular that the abundances in carbonaceous chondrites are very much higher than those in ordinary chondrites[4]. A. G. W. C. based his 1968 table of abundances[3] as much as possible on those in type I carbonaceous chondrites, or C1 chondrites, on the grounds that the more volatile elements are depleted relative to C1 abundances in all other types of chondrites. Thus the C1 meteorites have seemed to him to be the least fractionated source of abundances of the elements in the solar system apart from the Sun.

A spectrographic method[5] suggests an abundance of 126 for boron in a C1 meteorite. In four colorimetric analyses of two C1 meteorites, Mills[6] found an average abundance of boron of 5.7 parts per million, with a concentration range of 5.1 to 7.1 p.p.m., and a resulting abundance of 144. Using a fluorimetric technique, Quijano-Rico and Wanke[7] obtained an abundance of 186 for boron in one C2 meteorite, and an abundance of 121 in three C3 and C4 meteorites. A straightforward average of these values indicates a boron abundance of about 140. But the seeming constancy of these values of the boron abundance among the carbonaceous chondrites is inconsistent with the great depletion of boron in ordinary chondrites, as we shall discuss. This has motivated us to make a cosmochemical analysis of the condensation conditions for boron in a low pressure gas of solar composition, such as may have existed in the primitive solar nebula from which meteoritic materials condensed.

Because some of our discussion relating to the boron abundance in the Sun requires knowledge of the abundance of beryllium, we also discuss that abundance. Beryllium has an abundance much less variable in chondritic meteorites than boron, the average abundance in ordinary chondrites[2,3] being about 0.69. The preferred data on beryllium were published by Sill and Willis[8] who measured only one carbonaceous chondrite of type C2 and found an abundance of 0.81. This value has been adopted tentatively within the cosmochemical analysis we discuss here.

Condensation of Boron and Beryllium

Recent attempts[9-13] at predicting the behaviour of the elements during the condensation of the solar nebula have been based upon theoretical studies of the sequence of condensation of minerals from a cooling, low-pressure gas of solar composition. Be has been ignored in these thermodynamic calculations and the only work on B to date indicates it should not have condensed above 1,700 K at a partial pressure of hydrogen in the solar nebula of 0.5 atm (ref. 9).

Grossman[13] assumed that each condensate in the solar nebula remained in complete chemical equilibrium with the vapour, allowing a prediction of the variation of the principal element composition of the co-existing vapour with falling temperature. He solved the full equations only at temperatures greater than 1,200 K at 10^{-3} atm in that study, but we use the extrapolated vapour composition data at the lower temperatures required for B condensation, because less than 10% of the total condensable matter was left in the vapour state at 1,200 K. The presence of trace quantities of B and Be has a negligible effect on the principal element composition of the vapour.

Of forty-eight gaseous molecules containing boron for which thermodynamic data are readily available[14], only HBO_2, H_3BO_3, $NaBO_2$, and KBO_2 were found to be important in the temperature range of interest. Using a boron cosmic abundance of 140, we investigated the condensation temperatures of the crystalline phases KBO_2, HBO_2, H_3BO_3, $NaBH_4$, BN, $NaBO_2$, TiB, MgB_2, B_2O_3, TiB_2, $K_2B_6O_7$, MgB_4, $Na_2B_4O_7$, $K_2B_6O_{10}$, $B_{10}H_{14}$, $H_4B_2O_4$, and CB_4. Because of the relative stabilities of both gaseous and crystalline $NaBO_2$ and KBO_2, the principal difficulty in determining the condensation temperature of boron arises from uncertainty in the pressures of Na and K, two of the uncondensed elements at 1,200 K. Selecting a range of four orders of magnitude in each of the partial pressures of Na and K leads to a range of B condensation temperatures between 759 and 745 K (as $NaBO_2$) at a total pressure of 10^{-8}. These calculations assume that the most stable gaseous compounds and the highest condensing crystalline compounds of B in a solar gas have been considered and that B does not condense in solid solution at higher temperature.

We applied the same type of calculation to Be, assuming a cosmic abundance of 0.81. $Be(OH)_2$ and BeOH are the dominant gaseous species in the 1,200 to 1,800 K range. Be is almost totally condensed as $BeAl_2O_4$ in solid solution in spinel at 1,400 K. Again we assume that no important gaseous or crystalline species have been omitted from the consideration.

Many moderately volatile elements such as Mn, the alkalis, Cu, Au, Ga, and Ge exhibit abundance anomalies in the different classes of carbonaceous chondrites. In the C2 and C3 meteorites their concentrations, relative to Si, are all about 0.55 and 0.33, respectively, of their abundances in C1 meteorites. These relatively constant abundance depletions have been discussed in detail by Larimer[10,15] and Anders[15-17]. They have proposed that the carbonaceous chondrites are mixtures of fine-grained matrix material which remained in condensation equilibrium with the nebula down to ~350 K, and chondrules and metal grains which stopped equilibrating with the nebular vapour between 1,150 K and 1,250 K at 10^{-4} atm total pressure. The results obtained by Grossman[13] suggest that these temperatures should be 75 K lower. The low temperature matrix thus contains its full complement of the fractionated elements and the high temperature chondrules are devoid of them. The C1 meteorites are almost entirely matrix material and the average chondrule/matrix ratios in the C2 and C3 meteorites are in the ranges required by this model to account for the observed abundance depletions.

At 10^{-4} atm, Be would have condensed at about 1,325 K, somewhat above the maximum condensation temperatures, 1,075 to 1,175 K, of the fractionated elements. Thus, Be should not be fractionated among the carbonaceous chondrites and the abundance in the C1 meteorites must be very close to that in the C2 meteorites, 0.81. Boron, however, would have condensed at about 700 K at 10^{-4} atm and should, therefore, be classed among the volatile elements which are fractionated between the different types of carbonaceous chondrites. Indeed, abundance data[7] indicate that the ratio of boron in C2 to that in C3 and C4 meteorites is 1.54, which is within 10% of the average ratio, 0.55/0.33, observed for the other volatile elements. This suggests strongly that the abundance of boron in C1 meteorites could be calculated from the C2, and the C3 and C4 abundances, by dividing them by 0.55 and 0.33 respectively. yielding boron abundances of 339 and 367. If the boron abundance is increased by this much over that used in the condensation calculation, the condensation temperature is increased by only about 25 K, which is unimportant for the purposes of this computation.

It is thus our opinion that the boron abundances in C1 meteorites found by Harder[5] and by Mills[6] are too low, and we therefore adopt a value of 350 for the cosmic abundance of boron. We emphasize that it is highly desirable that this conclusion should be checked by better analyses of boron in C1 meteorites. For beryllium we adopt the C2 abundance of 0.81 as the cosmic abundance.

Boron in the Interstellar Medium

A tentative determination of the abundance of boron in the interstellar medium was made following a rocket ultraviolet measurement (A. M. Smith and T. P. Stecher, unpublished). In this work a feature at 1,362.46 Å in the spectrum of Zeta Ophiuchi was identified as interstellar boron II. A curve-of-growth analysis indicated a ratio of boron to oxygen in the interstellar medium of 1.9×10^{-5}. An oxygen abundance of 2.15×10^7 yields a boron abundance of 418. Although one must be cautious about accepting abundance determinations based on measurements of only one line, it is interesting that this boron abundance is in essential agreement with that which we deduced in the preceding section. It is to be hoped that this matter can be investigated further with Copernicus.

Abundances in the Sun

The situation concerning the abundance of beryllium in the Sun has been summarized recently by Hauge and Engvold[18]. Their abundance values have been converted to the silicon scale. The beryllium abundance is derived from the BeII resonance doublet at 3,131 A. One of the lines is seriously blended and should not be used for abundance analyses. Beryllium abundances in the Sun were found in 1968 to be 0.47 by Grevesse[19] and 0.29 by Hauge and Engvold[20], and in 1970 Muller obtained a still lower abundance of 0.20 (quoted in ref. 18). From this last value one would conclude that beryllium is depleted to 0.25 of its normal cosmic abundance in the material at the solar surface, although we emphasize that one must be very cautious in using abundances determined from only one spectral line.

The situation concerning boron in the Sun has also been discussed by Hauge and Engvold[18]. Boron has not been detected in the Sun. The strongest lines fall in inaccessible spectral regions. Grevesse[19] concluded that the boron abundance is less than 20 from the absence of two BI lines at 11,661 A. Engvold[21] concluded that the boron abundance was less than 10 following an unsuccessful search for the absorption band spectrum of the BH molecule in sunspots. We have checked to see whether any boron molecular species should be expected under the conditions in which sunspot spectra are formed, other than those considered by Engvold[21], and we conclude that no significant amount of boron can be hiding in some molecular form not considered by Engvold. From this it seems that boron in the surface layers of the Sun has been depleted by at least a factor of 35.

For comparison, we note that the abundance of lithium[22] in C1 meteorites is 49.5, which can be compared with the solar photospheric value[23] of 0.20. Thus the solar depletion factor is about 250.

There is a clear inconsistency among the solar abundances. Lithium, beryllium, and boron are all destroyed at hydrogen-burning temperatures in the central regions of the Sun. The rate of destruction of beryllium is intermediate between the rates at which the lithium and boron isotopes are destroyed. Therefore it seems necessary to conclude either that the one line in the solar spectrum from which the beryllium abundance has been obtained is really due to some other element, or that the boron abundance in the Sun is actually an order of magnitude greater than the upper limits which have been set by the several searchers for it. Because many different lines of boron and boron compounds have been searched for in the Sun, without success, we believe that the solar beryllium line is suspect and should be re-examined.

If we accept that beryllium and boron, as well as lithium, are strongly depleted in the solar surface layers, then an unexpected amount of mixing has taken place within the solar interior some time during the history of the Sun. Using the nuclear reaction rates tabulated by Fowler, Caughlan, and Zimmerman[24] we conclude that the destruction of beryllium and boron will occur on a time scale of 10^7 to 10^9 yr in the deep solar interior, provided the temperature is in excess of 5×10^6 K. In a model of the Sun which had been evolved without mixing for 4.6×10^9 yr, provided by D. Ezer, 75% of the mass in the interior is at a temperature greater than 5×10^6 K. Hence almost all of the outer 25% of the solar mass has been replaced at some time by material coming from a much greater depth.

This raises some interesting questions concerning the helium isotopes in the Sun. In the evolved but unmixed model of the Sun, the ratio of ^3He to ^4He rises steadily with increasing radial distance from the centre of the Sun, reaches a maximum at a temperature of 7×10^6 K at a point at which approximately half the mass is contained within the interior radial distance, and then decreases toward the low initial value in the solar surface layers. The maximum value of the ratio is 0.17, which is orders of magnitude greater than the ratio of the isotopes observed in the solar wind.

So mixing within the solar interior cannot have brought material from near the centre to the surface in recent times, or the ratio of the helium isotopes would be much greater in the solar wind that the observed values[25] which are about 4×10^{-4}. This suggests that the extensive mixing within the solar interior occurred rather early in its history, before the ^{3}He had had time to build up a substantial abundance following the onset of the proton-proton thermonuclear reactions. One possible mechanism which seems consistent with this requirement is the operation of Eddington-Sweet circulation currents in the Sun[26,27], which would be quite rapid if the initial Sun were spinning at an angular rate nearly sufficient to produce rotational instability at the equator. Such circulation currents would be shut off when the Sun developed a substantial gradient in the mean molecular weight following conversion of hydrogen to helium near the centre.

Because of these considerations we think it is dangerous to make the common assumption that the abundance of ^{3}He in the surface layers of the Sun represents the sum of the initial abundances of ^{3}He and deuterium in solar material. The abundance of ^{3}He may have been modified by the mixing process.

Boron Production by Cosmic Rays

One of the seemingly more successful theories of recent years has been that of Reeves, Fowler, and Hoyle[1], who found that cosmic ray bombardment of the interstellar medium could successfully account for the abundances of the light elements in the solar system. This theory, in its more recent refinements[28-31], seems to account quantitatively for the production of ^{6}Li, ^{9}Be, ^{10}B, and ^{11}B. It did not produce enough ^{7}Li to agree with the observed abundance of that isotope, but there are other possible astrophysical sources which can produce this[32]. The theory accounted for the older low values of the boron isotope abundances.

The great upward revision of the boron abundance requires modifications to this theory. We emphasize that the cosmic ray production theory continues to account quantitatively for the abundances of ^{6}Li and ^{9}Be. But an additional mechanism must be found which can produce the boron isotopes.

We note that Fowler, Reeves, and Silk[28] have argued that a large flux of low-energy cosmic rays ($\sim$5 to 10 MeV) could not exist in sufficient quantity to heat the interstellar medium, because this flux would have produced too much boron. The new boron abundance is so much higher than the old one that this restriction on heating of the interstellar medium by a hypothetical flux of low-energy cosmic rays is now removed. Instead, one should ask whether the hypothetical flux of low energy cosmic rays might be the necessary mechanism to produce the additional boron.

Boron-10 is produced by the reaction ^{13}C(p,α)^{10}B, which has a Q value of −4.063 MeV. Boron-11 is produced by the reaction ^{14}N(p,α)^{11}C, which has a Q of −2.922 MeV. The abundances of ^{13}C and ^{14}N in the interstellar medium seem to have the same relative values as in the solar system[33]. Thus, in order to produce the solar system ratio of the boron isotope abundances, it is a necessary condition for the validity of the low-energy cosmic-ray bombardment hypothesis that the cross-section for the production of ^{10}B from ^{13}C, properly averaged over a cosmic-ray bombardment spectrum, should be higher than that for the production of ^{11}B from the proton bombardment of ^{14}N by a factor of 6.8. D. Bodansky provided unpublished cross-section data on the reaction ^{13}C(p,α)^{10}B and J. W. Truran informs us that, according to these data, the ratio of the average cross-sections for the two reactions is much less than 6.8.

Thus we conclude that it is no longer possible, from boron abundances, to argue that the heating of the interstellar medium may not occur primarily from a flux of low-energy cosmic rays. Such a flux may contribute significantly to the production of the boron isotopes, but we conclude that it is not the principal contributor to the production of these isotopes.

Boron Production from Supernovae

One of us[34] has recently prepared an extensive analysis of the structure of shock waves in supernova explosions, demonstrating that this structure may give rise to the production of the deuterium in the Galaxy through spallation of the helium contained in the outer parts of the supernova envelope. A substantial production of the boron isotopes should also occur under these circumstances. We give a brief account of the analysis here.

If a supernova explodes as a result of a nuclear detonation process in which about 1 MeV per nucleon is produced, and this supernova contains an extended atmosphere of hydrogen, essentially in a red giant configuration, then as the supernova shock wave progresses through this atmosphere, the rapidly decreasing density in the envelope will result in a strengthening of the shock wave. If the only constituent in the material traversed by the shock wave were the ions, the temperature of the ions behind the shock front would become progressively higher as the shock wave strengthens. Thus one would typically get ion temperatures of several MeV near the surface of the supernova envelope.

In fact, the energy behind the shock is shared between ions, electrons, and photons, and because of the low density most of the energy goes into the photons. The typical temperature behind the shock will be closer to one keV. But this asymptotic condition can only be approached gradually, because a very large number of photons must be created for each ion present, and these photons must then be heated.

The actual shock process starts with a heating of the ions in the shock front precursor by collision between the ions of the stationary gas and the ions moving in the shock surface. These collisions raise the ion temperature to several MeV. Energy is now fed from the ions to the electrons, which are heated to a temperature of about m_ec^2. Collisions between the electrons and the ions create photons by the bremsstrahlung process; most of the photons have very low energy. Collisions between the electrons and the low energy photons raise the average energy of these photons. The ion energy is thus channelled through the electrons into photons and, as the ion temperature gradually falls, the temperature of the electrons will eventually also fall as the system approaches thermal equilibrium.

The quantitative estimate indicates that it takes about 140 Compton collision periods for the initial high ion temperature to drop by about a factor of 2. During this time the collisions among the ions have brought about an approach toward a Maxwellian energy distribution; the distribution should be fairly accurately Maxwellian to about $4kT_i$, where T_i is the ion temperature, and beyond this there will be a gradual depletion of the high energy tail relative to the Maxwellian distribution. An ion temperature of about 10 MeV is sufficient to spallate most of the helium present in the medium, producing deuterium both directly and by way of the production of neutrons which are captured on the protons of the medium following the thermalization process.

The production of the boron isotopes from ^{13}C and ^{14}N has much lower spallation thresholds than that for production of deuterium from helium. Therefore we expect the production of the boron isotopes to take place at a much lower ion temperature than that at which the deuterium production takes place. This is fortunate, for otherwise the ^{10}B would be severely depleted by the neutrons that are produced in the spallation of helium. The ^{13}C and ^{14}N target nuclei may or may not have been enhanced in the outer envelope of the supernova during the preceding evolution of the star.

The situation is much more complex than in spallation processes taking place in the interstellar medium. The ion temperature remains high for a long enough period so that multiple nuclear reactions can take place on each affected nucleus. A reaction which is of obvious potential importance is $^{10}\mathrm{B}(p,\alpha)^{7}\mathrm{Be}$, which is exothermic with a Q value of 1.146 MeV. If, as we believe, this supernova production mechanism has been primarily responsible for the production of the boron isotopes in nature, then this secondary reaction may be primarily responsible for the production of ^{7}Li, which is formed following electron capture on ^{7}Be.

All of the product nuclei, ^{7}Be, ^{10}B, and ^{11}C, may be destroyed by further spallation reactions. Because it is possible that only a fraction of the produced material survives following the thermalization of the shock wave, because the cross-sections for destruction of the product nuclei are in general not known, and because the abundances of the target nuclei are uncertain, we do not feel in a position to predict the relative production of ^{7}Li, ^{10}B, and ^{11}B which results from the supernova shock process. The abundances of these product nuclei relative to the target nuclei, ^{13}C and ^{14}N, in the solar system, are of the same order as the initial deuterium abundance in the primitive solar nebula relative to the solar helium abundance. Thus it is not quantitatively implausible that the abundances of deuterium, ^{7}Li, ^{10}B, and ^{11}B, should all be produced in their solar system proportions in these supernova shock waves.

The review article on the meteoritic abundances of boron by Baedecker[4] first drew our attention to the necessity for this upward revision in the cosmic abundance of boron.

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Emission Mechanism in Pulsars

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In a new model of the emission from pulsars based on the relativistic beaming effect, the source of the radiation is the circular motion of high energy electrons round magnetic field lines. The radio emission at the gyrofrequency is from coherent bunches of electrons, and the optical and X-ray emission is the incoherent synchrotron radiation from the same electrons.

ALTHOUGH there is as yet no agreement on the location of the source of the pulsed radiation within the magnetosphere of a pulsar, there is now considerable evidence that it is located close to the velocity of light circle, so that the corotation of the magnetosphere moves the source with a velocity of the order of 0.8 to 0.9 *c*. The pulses are then formed by the process of relativistic beam compression[1–5], which allows the radiation process to be isotropic within the local frame of reference within the magnetosphere, giving a radiation beam solely due to the geometrical movement of the whole source.

Here I discuss the nature of the radiating source which the observations would require if the beaming effect is indeed primarily due to relativistic beam compression. I shall show that a simple and self-consistent model can explain the whole of the electromagnetic radiation from the Crab Nebula pulsar, including some of the more detailed characteristics of polarization and variability. The lack of optical pulses from other pulsars is also explained simply, as indeed it is on all other models so far put forward.

Characteristics of the Emitter

Although the Crab Nebula pulsar is the only pulsar known to radiate outside the radio spectrum, there is no reason to suppose that its radio emission differs basically from that of other pulsars, for which the radio emission has been studied in greater detail. One may therefore list the following features of pulsar emission which must be explained by any comprehensive model: (i) the integrated pulse envelope; (ii) the individual sub-pulses; (iii) the microstructure, appearing as sharp pulsations within a single sub-pulse; (iv) the polarization structure in the integrated pulse envelope; (v) the polarization of the sub-pulses; (vi) the variability of the radio pulses; (vii) the lack of variability in the optical pulses; (viii) the rapid decrease of the radio spectrum with frequency; (ix) the flatness of the optical spectrum and the decreases both in the infrared and the ultraviolet; (x) the extension of the spectrum to X-ray and gamma-ray frequencies; (xi) the small variation of the integrated pulse envelope over the whole electro-magnetic spectrum, that is, over more than fifty octaves; (xii) the evidence of a narrow-band character in the microstructure of pulses; and (xiii) the extremely high volume emissivity both in the radio and in the optical and X-ray spectrum.

I shall discuss these properties in the light of a proposed model which seems to be capable of accommodating all of them.

Model Emission Mechanism

I suggest that the radio emission is from bunches of electrons gyrating round the magnetic field within a small range of longitude defined by a particular field configuration. The radio radiation is coherent at the gyrofrequency and is narrow band. The same electrons, which have relativistic energies, radiate broad-band synchrotron radiation at high harmonic frequencies, that is, in the optical and X-ray spectrum. This harmonic radiation is not coherent.

As the radio emission is narrow band, the observed wide radio spectrum must correspond to a distribution in resonant frequency of the narrow-band sources. This distribution must also be a spatial distribution, so that different parts of the source have different resonant frequencies. The optical and X-ray spectrum will then be determined largely by the corresponding distribution of synchrotron critical frequency, which can be regarded as a combination of gyrofrequency and particle energy.

This model is distinguishable from most others in three ways which I shall discuss in the following sections: (i) the radio emission is narrow band, (ii) the pulse width is related to the relativistic movement of the source rather than to a radiated polar diagram; and (iii) optical and X-ray emission can occur from the same particles as those which are emitting radio waves.

Spectra of Pulsars

Published radio spectra[6] refer to the total energy in the whole pulse, averaged over many pulses. These spectra show a monotonic steep fall with frequency above about 300 MHz for all pulsars, with spectral indices generally between -2 and -3. Below 300 MHz many spectra become flatter, and some show a reverse slope below 100 MHz.

These spectra have generally been interpreted as emission from a broad-band source. There is, however, considerable evidence that the basic radio emission process is in fact narrow band, so that the broad spectrum represents the sum of emission from an ensemble of narrow-band sources.

The principal evidence is as follows:

(i) The integrated profile for many pulsars is dependent on radio frequency. If this profile represents a spatial distribution of sources, then the dependence must be interpreted either as large differences in the spectra of moderately broad-band sources, or more probably as a relation between frequency and position of narrow-band sources. In pulsars such as PSR 1133+16, some parts of the profile have a very steep slope, corresponding to a spectral index of -5. An even steeper slope has already been reported for the "precursor" component of the Crab Nebula pulsar[7]. These steep slopes suggest a high frequency cutoff, more consistent with an end to the range of narrow-band sources than to a sharp spectral feature in the radiation from a single broad-band source.

(ii) The "microstructure" of pulses from PSR 0950+08, as observed at 408 MHz, has a bandwidth of only about 20 kHz (ref. 8); this is not far from the inverse of the microstructure duration, suggesting that the sources of individual pulses are basically monochromatic but with a rapidly changing excitation.

By contrast, individual sub-pulses usually have large bandwidths[9]. The narrowest known frequency structure here is in the giant pulses from the Crab Nebula pulsar, which are often unrelated at frequencies as close as 74 and 111 MHz (ref. 10). The simplest interpretation seems to be that the sub-pulses contain narrow-band emission excited simultaneously, or nearly simultaneously, from sources covering a wide range of frequency.

Pulse Width and the Polar Diagram

It is part of the hypothesis of relativistic beam formation, which is adopted here, that no part of the time structure of the pulses need be identified with a beam formed in the emission process itself. If there were any large directivity in the emission process, then the sub-pulses would be correspondingly reduced in width. Because the directivity of any emitter can be expected to vary with wavelength, the sub-pulse width would then vary with radio wavelength, which is contrary to the available evidence. I therefore assume that the emission polar diagram is wide; it may even possibly be isotropic. Furthermore, a single sub-pulse presents a cut through this wide polar diagram covering about 180°, the cut being compressed into a pulse by the relativistic effect.

The clearest distinguishing feature of the sub-pulses is that they are, as individuals, typically 100% polarized[11]. The changes of polarization during a sub-pulse can therefore be represented by the movement of a point on the surface of a Poincaré sphere, which is a surface on which the two hands of circular polarization are represented by points at the poles, linear polarizations with various position angles are represented along the equator, and elliptical polarizations appear at intermediate latitudes depending on their ellipticities.

The observed changes of polarization within a sub-pulse correspond to a smooth movement over the Poincaré sphere of up to half of one circumference, at an arbitrary position angle. For example, the hand of polarization often changes once, but never twice, during a single sub-pulse; again the position angle often changes through a right angle, but seldom more unless there is a large degree of circular polarization.

Allowing for relativistic beam compression, this result must be interpreted as an emission pattern which includes all possible elliptical polarizations, from plane to circular, varying smoothly over a wide range of angles, probably reaching 180°. It has already been remarked[1] that this is a good description of the polar diagram of cyclotron radiation, and that no such pattern can be produced by curvature radiation from particles streaming along field lines.

Synchrotron Emission

The electrons gyrating in bunches round the field lines may have high relativistic energies and they will therefore radiate synchrotron radiation. The radiation will be incoherent for wavelengths smaller than the bunch size, so that the radiated power is simply proportional to the number of electrons. The large variations in the radio pulses are then seen to be variations in the bunching process, which leaves the optical emission constant, in accordance with observation[12].

Synchrotron radiation is beamed in the direction of particle motion. A single electron with energy γ gives a beam width of $1/\gamma$ at frequencies close to the critical frequency; the beam is fully polarized, generally elliptically but plane polarized at the maximum. The observed optical polarization of the Crab Nebula pulsar is linear, approximately 10%, and varying through the pulse. This is evidently explicable as the sum of synchrotron radiation from an ensemble of sources, spread in longitude. Such an ensemble would average out the circularly polarized components, and reduce the plane polarization.

The synchrotron spectrum of a single electron is expected to be flat in the region of the critical frequency, falling exponentially at higher frequencies, and varying as $\nu^{1/3}$ at lower frequencies. The ensemble of sources may have a spread in particle energy, and hence of critical frequency; there may also be a low frequency cutoff due to self-absorption.

The observed spectrum of the Crab Nebula pulsar is flat in the optical region, which may tentatively be identified with the flat region just below the critical synchrotron frequency.

Numerical Values

The radio volume emissivity, averaged over a sub-pulse, has previously been estimated on the basis that the emitting volume occupies about 0.05 of a sphere which extends out to the velocity of light circle, that is, $0.05(4/3)\pi(c/\Omega)^3$. There is no real basis for taking any other volume, although it is to be expected that the actual volume may be considerably smaller. The recent observations[13,14] of intense sub-pulses now suggest an increase of volume emissivity of a factor of 40 for PSR 0950+08, so that typical values for volume emissivity at radio wavelengths are 10^8 W m^{-3} for PSR 0521+22 and 10^4 W m^{-3} for PSR 0329+54. Both these refer to a bandwidth of 100 MHz at a centre frequency of 100 MHz. The emissivity is assumed to change with the short time scale observed by Hankin.

The power P_c radiated by a single electron at its fundamental cyclotron frequency ν, whatever its energy, is

$$P_c = (8\pi^2/3)(e^2\nu^2/c^2)$$

Typically I consider radiation observed at 200 MHz, which is radiated at about 50 MHz and Doppler shifted due to the motion of the whole source. Then

$$P_c = 5\times10^{-20} \text{ W}$$

Suppose the volume density of radiating electrons is N m^{-3} and that all electrons are bunched over a volume of about 1 m^3, giving full coherence at the typical frequency of 50 MHz. Then the volume emissivity is $5\times10^{-20}\ N^2$ W m^{-3}, or somewhat less depending on the precise configuration of the bunch. So for PSR 0532, $N=5\times10^{13}$ m^{-3}, and for PSR 0329, $N=5\times10^{11}$ m^{-3}.

The cyclotron frequency ν_H is determined by the field H (gauss) and the electron energy γm_0c^2, so that $\nu_H=2.8\ H/\gamma$ MHz. For the outer part of the magnetosphere of PSR 0532 the field is believed to be 10^5 to 10^6 gauss, corresponding to a surface field of 10^{12} gauss. I deduce that $\gamma=5\times10^3$ to 5×10^4. For other pulsars the field is expected to be less, falling as the inverse cube of the period if the surface field is the same. We must therefore suppose that γ also decreases with increasing period, keeping the cyclotron frequency within the radio frequency band. I return to this point later.

A single, gyrating, highly relativistic electron radiates a total power

$$P_T = (8\pi^2/3)(e^2\nu^2/c)\gamma^4$$

Most of this power is emitted at frequencies in the region of the critical frequency $\gamma^3\ \nu_H$. The bunches of electrons which emit strongly enhanced radiation at the fundamental frequency ν_H will emit incoherently at these high harmonics, so that the volume emissivity will be NP_T. Using the adopted values for ν_H and γ, the critical frequency for PSR 0532 is between 10^{17} and 10^{20} Hz, that is, in the ultraviolet to X-ray region. Below the critical frequency the spectrum is expected to be flat, ultimately falling as $\nu^{1/3}$ at lower frequencies (unless there is an additional effect of self-absorption). The total power emitted depends on γ^4, and must therefore be uncertain. But with $M=5\times10^{13}$ m^{-3} the observed volume emissivity of 3×10^{12} W m^{-3}, mostly in the ultraviolet and X-ray region, would be obtained for $\gamma=3\times10^4$, within the range of the expected values.

This simple analysis suggests that the coherent radio and the incoherent optical and X-radiation could be emitted by the same electrons. This is the chief conclusion of the analysis.

Implications

The analysis has so far proceeded from the observed characteristics of the radiation, without regard to any model of the pulsar magnetosphere or to the expected behaviour of charged particles within the magnetosphere. I now discuss the chief problems in relating the radiation model to our present understanding of the magnetosphere.

(i) Is there a preferred location of the emitter? The stability of the pulse periodicity implies that the source must lie on certain preferred lines of force. F. D. Kahn has suggested (private communication), and some observations confirm[11], that the lines of force are those which point forwards of the radius vector at the velocity of light cylinder. These occupy a suitably restricted longitude range, and they are lines on which the particles are expected to attain high energies.

(ii) Is cyclotron motion reasonable? As a first approximation charged particles move along the lines of force, with very small pitch angles. This cannot continue indefinitely along the lines which point forwards at the velocity of light cylinder; either the particles must break away or the pitch angle must increase. There seems to be a possibility that the pitch angle will approach 90° at a particular point along such lines of force.

(iii) Coherence and bunching. An instability leading to bunching has been described by Goldreich and Keeley[15] who also discuss the conditions for coherent synchrotron radiation from the bunches. Their analysis refers to electrons constrained to move in a ring, all with the same energy. In the pulsar magnetosphere there must of course also be an energy supply, presumably in the form of an electron stream.

(iv) Particle lifetime. In the Crab Nebula pulsar the particle energy density deduced from the radio emission can only sustain the optical and X-ray emission for about 10^{-5} s. Although it seems possible for fresh particles to enter the emitting region at a sufficient rate, it is also possible that the incoherent radiation is produced by lower energy particles over a larger volume, with a correspondingly longer lifetime.

(v) Long-period pulsars. No obvious dependence of radio spectrum on period P has yet been found, so that the theory must allow for coherent cyclotron radiation at frequencies higher than 50 MHz for all known pulsars. Because for a dipole field the strength varies as r^{-3}, the field at the emitting region is expected to fall as P^{-3}, so that it is 10^6 smaller for the long period pulsars than for the Crab Nebula. In this model of the Crab Nebula pulsar I found $\gamma\approx3\times10^4$, so there seems no possibility of obtaining sufficiently high cyclotron frequencies in all pulsars unless either the frequency is increased by Doppler shift due to velocity along the field lines, or the field strength is increased. Doppler-shifted cyclotron radiation would not show the correct polarization properties, as it emphasizes only one hand of circular polarization. I must therefore suggest that the field strength in the long period pulsars is greater than in the Crab Nebula pulsar by a factor of 10^2 or 10^3. This may account for the relative lack of long-period pulsars, for only those with unusually large fields would in fact radiate at radio frequencies.

The simple calculations here show that the same electrons giving rise to the coherent radiation at radio frequencies could account for the radiation at optical and higher frequencies. The radio frequency radiation is narrow band, so that a full theory must include an ensemble of sources over a range of fundamental frequencies. This range must correspondingly widen the optical and X-ray spectrum, which need not show the exponential fall above a single critical frequency which is typical of synchrotron radiation.

Further elaboration of this theory could account for the variations in the integrated pulse profile, from low radio frequencies to gamma rays, in terms of small variations of field and electron energy in different parts of the source.

The absence of optical radiation in pulsars other than the Crab Nebula pulsar is understandable as a combined effect of lower electron energy and lower magnetic field. The comparatively small population of pulsars with long periods may possibly be those with the strongest magnetic fields.

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Intense Vibrational Absorption Spectra of Trapped Triatomic Radicals

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Intense spectra of triatomic radicals have been produced by means of a surface reaction of hydrogen atoms, formed by a microwave discharge in flowing gas, with diatomic oxides. Failure of this method to produce detectable formyl radicals is attributed to existence of an activation energy greatly exceeding 200 cm^{-1} for reaction of H with CO. New NH and ND fundamental vibrations of HNO and DNO, at 2,719 and 2,043 cm^{-1}, are now consistent with the known large bond length. The weak absorption due to the bending mode of DO_2 has at last been located at 1,085.3 cm^{-1}, enabling a vibrational assignment for hydroperoxyl radical to be completed.

Although much quantitative structural information has been obtained on the free radicals formed by reacting hydrogen atoms with CO, NO or O_2 to form formyl, nitrosyl hydride or hydroperoxyl radicals, certain peculiarities remain. Missing vibrational fundamentals, anomalous bond-length-wavenumber correlations and activation energies for the formation reaction are some of these properties. I have extended the electric discharge method of Dewar[1], as developed by Geib and Harteck[2,3], to produce very intense vibrational absorption spectra which have provided information on these outstanding problems.

The basic apparatus is shown in Fig. 1. Gases from a standard high-vacuum manifold passed to 'Cryotip' refrigerators operated at 17 K, mounted in 'Unicam SP100G' or 'Perkin-Elmer 225' spectrometers. The quarter-wave cavity was fed with 75–125 W power at 2.45 GHz from a 'Microtron' generator to maintain a stable discharge in gas at 20–30 N m^{-2}. Both inlet systems operated with comparable efficiency. Hydrogen or deuterium (mol fraction 1–4% in argon) was discharged, whereas the oxide (CO, NO or O_2, 5–20% in Ar) was not.

When H atoms were reacted with molecular oxygen, very intense absorption at 1,389.0, 1,101.0 and another fairly intense absorption at 3,410.5 cm^{-1} were observed (Fig. 2*A*). When D atoms were used instead, intense absorptions appeared at 1,020.2 and 2,529.7 cm^{-1} (Fig. 2*B*). All these wavenumbers agree conclusively with those measured previously in photolysis experiments[4-6] in which hydroperoxyl product absorptions were much less intense. To complete the assignment, careful scrutiny of several minor absorptions was necessary. Characteristic absorptions of H_2O, H_2O_2, D_2O, D_2O_2 and O_3 were readily recognized. I believe the ozone observed to be derived from reaction of oxygen atoms, produced by bombardment of the silica tube in the discharge region by energetic argon ions, with oxygen molecules. (Correspondingly CO_2 and NO_2 were observed in experiments with CO and NO.) Weak absorptions at 830 and 760 cm^{-1} may be attributed to D_2O_4[7], presumably formed by combination of DO_2 radicals. All these absorptions were of variable intensity relative to the 1,020 cm^{-1} line of DO_2 in various experiments, whereas the 1,085.3 cm^{-1} line had an intensity ratio constant within experimental error. Also notable is a linewidth phenomenon. For all three fundamentals of HO_2 and the other two of DO_2, widths at half maximum intensity were always significantly greater than those of other species detected in the same spectral scans, such as H_2O, D_2O, O_3. The 1,085.3 cm^{-1} absorption shares this greater linewidth property. Thus both intensity ratios and linewidths support assignment of this line to DO_2.

This assignment can terminate a controversy between earlier predictions of the order of fundamentals, which generally placed ν_3 (O–O stretching) at greater wavenumbers than ν_2 (bending) and previous incomplete data from photochemical experiments[4]. The only reasonable assignment now consistent with all experimental data places ν_2 of HO_2 above ν_3. The intensity differences, small between ν_2 and ν_3 of HO_2 but large for DO_2, are similar to those for HCN and DCN vapours.

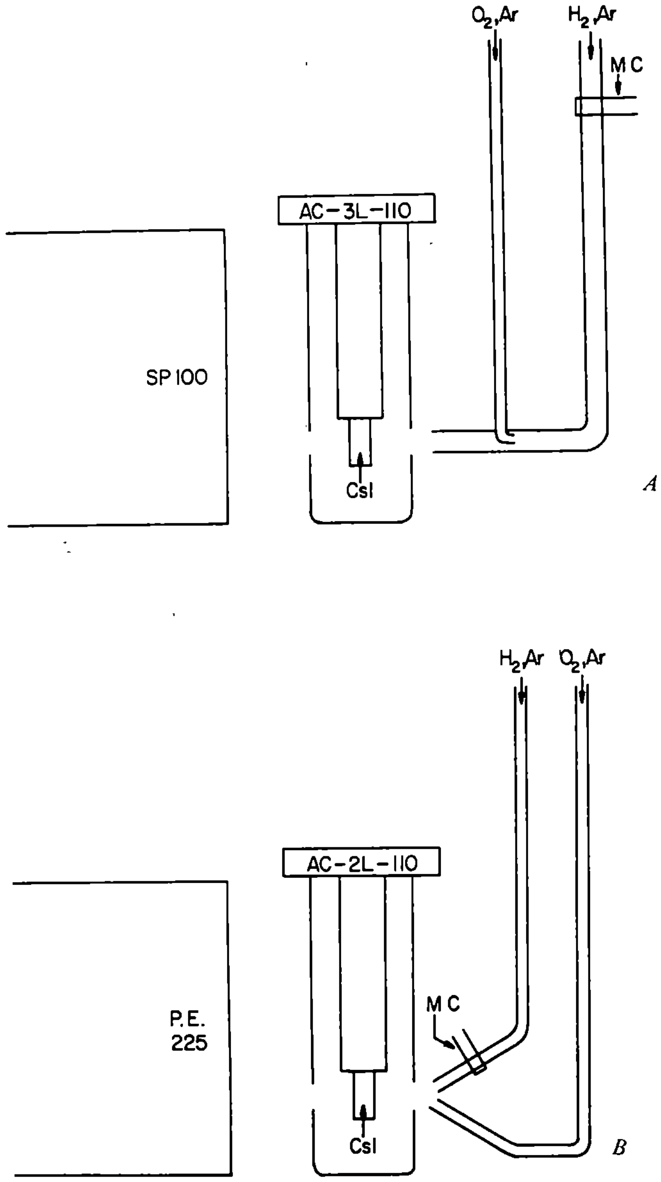

Fig. 1 Diagrammatic representation of inlet systems used to obtain intense spectra. MC, microwave cavity.

When H atoms were reacted with nitric oxide, no absorption in the 3,200–3,600 cm^{-1} was found to obey the intensity criterion, a consistent absorbance ratio with the characteristic 1,564 cm^{-1} feature of HNO, in experiments with varied conditions. A line at 2,719 cm^{-1} did, however, obey this criterion, and one correspondingly at 2,043 cm^{-1} for DNO, as shown in Fig. 3. These lines can be attributed to no other species known to be present, such as nitrogen oxides, nitrous acid, etc. The isotope wavenumber shift is as expected. As these NH and ND fundamentals are considerably weaker than the NO fundamentals, the intense spectra made possible by the flow discharge method were invaluable for these assignments. Furthermore the isolation of HNO or DNO molecules, surrounded by only argon atoms in most sites, prevents intermolecular interactions which could lead to wavenumber shifts. When HNO is produced on photolysis of methyl nitrite or nitromethane (unpublished results) for instance, the carbonyl fundamental of cage partner H_2CO is significantly displaced from the wavenumber for unambiguous isolation in other experiments; to expect a corresponding perturbation of HNO absorption wavenumber is entirely reasonable.

When H or D atoms were reacted with carbon monoxide under similar conditions, no trace of HCO or DCO was obtained in these flow discharge experiments, but minor absorptions due to impurities, as well as major absorption of CO, were easily detected.

The spectra in Figs. 2 and 3 make clear that this method can make possible relatively concentrated samples of matrix-isolated radicals. Consideration of gas pressure, both in the reaction zone and downstream, and available time, between mixing of reagents and freezing on the CsI substrate, precludes the reaction of, for example, H with O_2 in the gas phase; known specific velocities from gas kinetic studies and the requirement of a third body (M), $H+O_2+M\rightarrow HO_2+M$, mean that such a homogeneous reaction is impossible with either reactor in Fig. 1. Therefore the synthetic reactions must occur at or just below the surface layers of the deposit as it builds up while incident gases freeze thereon. The efficiency of production of radicals to produce intense spectra in short deposition periods (2–4 h) with small amounts of reactants means that mobility on the freezing surface must be possible, but also the relative scarcity of radical dimers (such as D_2O_4) indicates only a limited mobility. Consistent with these results is the hypothesis that H or D atoms are very mobile species, even when having only 200 cm^{-1} of kinetic energy (thermal energy for 300 K), so that they can diffuse across and into the argon surface layers for some brief period. Of course the surface layers at time of formation cannot be considered to be representative of the bulk of the sample. Proof that reaction occurs heterogeneously is provided in other experiments in which H or D atoms were reacted with NO_2; observed products included s-*cis* and s-*trans* rotamers of HONO but no trace of OH radicals. In the gas phase, the rapid bimolecular reaction[8] $H+ONO\rightarrow OH+NO$ is known to be much more efficient than the termolecular combination reaction $H+ONO+M\rightarrow HONO+M$. But even

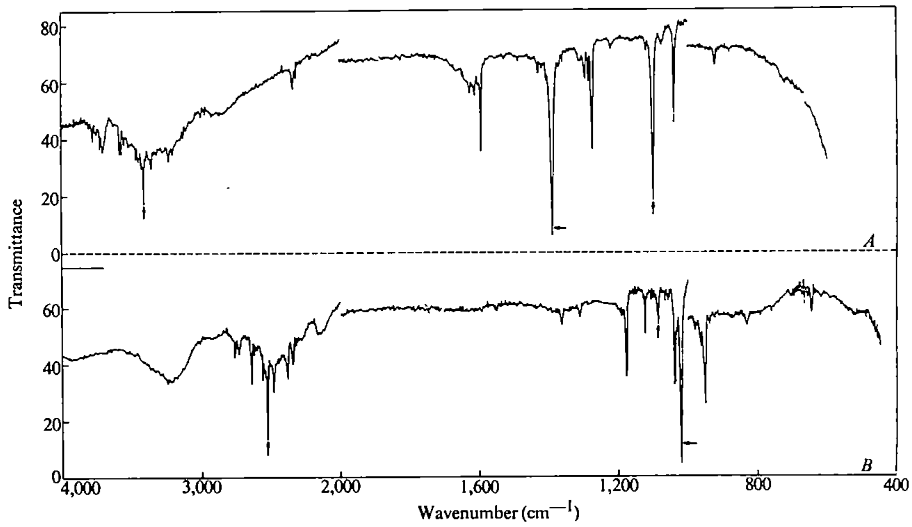

Fig. 2 Infrared absorption of products of reaction of (*A*) H and (*B*) D atoms with molecular oxygen. Arrows mark features attributed to HO_2 and DO_2.

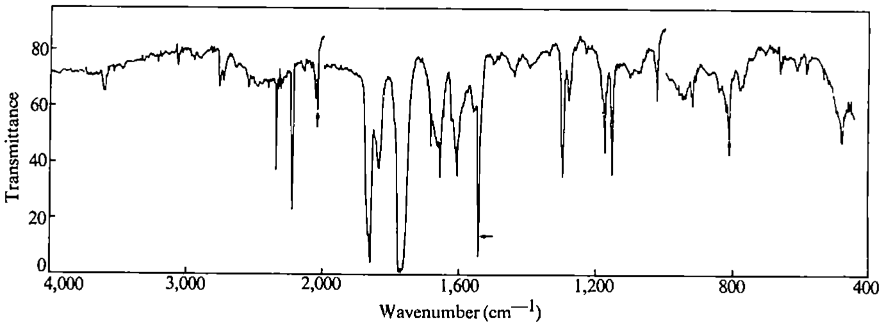

Fig. 3 Infrared absorption of products of reaction of D atoms with nitric oxide. Arrows mark features attributed to DNO.

H or D atoms can be trapped in argon; the absorption at 643 cm^{-1} in Fig. 2*B* is probably due to trapped D[9]. No absorption at 905 cm^{-1} is seen in Fig. 2*A*, but the scanning procedure used may have precluded observation[10].

Lack of formyl radicals in these experiments is positive proof of an activation energy exceeding 200 cm^{-1} (2.4 kJ mol^{-1}) for the synthesis reaction. Earlier evidence[11] for such an activation requirement was ill-founded[4]. When hydrogen iodide was photolysed (39,413 cm^{-1}) as a source of hydrogen atoms[4], 14,800 cm^{-1} of excess energy remained after 24,600 cm^{-1} was used in dissociating HI into ground-state atoms[12,13]; most of this excess energy must, by conservation of momentum, be left to the hydrogen atom as kinetic energy. The energy of activation of self-diffusion of metals is often estimated[14] to be two-thirds the energy of sublimation. If motion through the argon lattice required such an energy, 430 cm^{-1}, per step, and if almost all the excess energy available from that photodissociation had to be dissipated in this manner, H or D atoms could travel many steps from the site of production. Alternatively, a number of such translational steps could still occur while leaving the diffusing atom considerable energy for reaction initiation purposes. Radical concentrations attained in photochemical experiments suggest that the average transfer of "hot" hydrogen atoms consists of only a short motion. On the other hand, the quantity 430 cm^{-1} may be an underestimate, as infrared radiation at 905 cm^{-1} seems far more effective in causing loss of hydrogen atoms, presumably eventually by recombination, than at 644 cm^{-1} (for D atoms)[9]. In any case, we conclude that the flow discharge method is effective in producing radicals by synthesis only when at most a very small activation energy is required.

The vibrational and structural parameters of all three radicals are collected in the table for purposes of comparison. The structure of the formyl radical, previously reconsidered because of the vibrational spectra of trapped radicals[4] but calculated purely from gas-phase rotational constants[15], has now been accepted[16]. An alternative procedure[17], based on minimization of error in calculated vibrational wavenumbers, lacks physical justification. For nitrosyl hydride, previous assignments of the HN stretching fundamental, 3,300–3,600 cm^{-1}, were severely inconsistent with the large known bondlength and small dissociation energy (upper limit of 17,000 cm^{-1}), according to Bernstein's correlations[18]. This discrepancy has now been resolved satisfactorily on the basis of the new assignments.

Table 1 Structures and Fundamental Vibrations of Triatomic Radicals

	HCO	DCO	HNO	DNO	HOO	DOO
r_0(X-H), pm	114.5*		106.28†		98.5	
r_0(X-O), pm	117.65		121.16		136	
angle, degrees	123.3		108.58		106	
v_1, cm^{-1}	2,482	1,926‡	2,719	2,043	3,410.5	2,529.7
v_2, cm^{-1}	1,088	850	1,122	811.7	1,389.0	1,085.3
v_3, cm^{-1}	1,861	1,803	1,564	1,547.3	1,101.0	1,020.2

* Results from ref. 3.
† Results from ref. 14.
‡ Results from ref. 10.

The structure of HO_2 has been newly estimated on the basis of the definite vibrational assignment; the OH distance is derived from Bernstein's correlation[18], the OO distance from Orville-Thomas's correlation[19], whereas the interbond angle is based on theoretical results but is consistent with rough rotational constants estimated for HO_2 [20]. A complete normal coordinate analysis will be published elsewhere.

Finally, the alleged absorption of trapped hydroxyl radicals[21] (3,452.3 and 3,428.2 cm^{-1} for OH, or 2,543.2 and 2,526.2 cm^{-1} for OD) is inconsistent with both earlier direct observation[22] (at 3,574 or 2,635 cm^{-1}) and combination differences (of 3,573 for 2,634 cm^{-1}) in fluorescence spectra[23] of radicals trapped in neon.

Absorption spectra in the latter experiments are closely related to those of OH or OD in the gas phase, so there can be no doubt of the identity of the carrier. Purported spectra[20] of hydroxyl radicals are almost certainly due to hydroxyl anions, OH^- or OD^-. The method of production, vacuum ultraviolet photolysis during deposition, is known to lead to anions in other cases; for example, C_2^- has been obtained from HCCH in this manner[24].

I am indebted to the late Dr K. B. Harvey for acquainting me with the problems of these radicals. Some experiments in Cambridge University Department of Physical Chemistry were conducted during tenure of a Research Fellowship of Emmanuel College. Research support at Memorial University was provided by the National Research Council of Canada and the Research Corporation (New York).

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LETTERS TO NATURE

PHYSICAL SCIENCES

Strontium Isotopes and Rare Earth Elements in Basalts from the Heimaey and Surtsey Volcanic Eruptions

THE Vestmannaeyjar archipelago has been the site of two recent volcanic eruptions (Fig. 1). The first was the Surtsey eruption from November 1963 to June 1967 and the second is the Heimaey eruption which began on January 23, 1973, and is still continuing[1,2]. The first products of the 1973 Heimaey eruption are close to mugearite in composition. A gradational change in composition from mugearite to hawaiite was recorded during the first week of eruption[2]. Reaction between the mugearite magma and gabbroic xenoliths to form kaersutite and pargasitic hornblende[2] suggests fairly high f_{H_2O} in the magma.

The Vestmannaeyjar are almost exclusively made up of basalt lavas and tuffs of mild alkaline character, like the Surtsey lavas and the Helgafell lava[2]. Basalts from the Vestmannaeyjar, together with basalts from the Snaefellsnes area of western Iceland (Fig. 1), are the only examples of postglacial alkali olivine basalts in Iceland[3]—most postglacial basalts are tholeiites and olivine tholeiites. Postglacial transitional alkali basalts[3], however, occur in the Katla and Eldgja-region in southern Iceland. The areal distribution of the postglacial petrological provinces in Iceland[3] is shown in Fig. 1.

Here we compare the $^{87}Sr/^{86}Sr$ ratios in lava samples erupted in 1964 and 1966 in Surtsey with those in samples of both the present, and earlier, eruptions on Heimaey, in order to see if the ratios of their source regions differ in space and time or with chemical composition of lavas. The samples of older volcanic products on Heimaey include a Recent lava in Helgafell (the mountain which was cut by the 1973 fissure), a subglacial volcanic plug in Háin, Heimaey, and a subglacial volcanic plug in Dalfjall, Heimaey (Table 1). In addition to the Sr isotope measurements, measurements of rare earth elements in three samples are reported.

Table 1 Samples Analysed

Sample	Lithology	Time of eruption
VE-112	Mugearite tephra	January 23, 1973
VE-111	Mugearite lava	January 23, 1973
I-42	Alkali-olivine basalt	1966
I-43	Alkali-olivine basalt	1964
VE-67	Porphyritic alkali-olivine basalt	5,500 yr BP
VE-72	Alkali-olivine basalt	Subglacial
VE-93	Hawaiite plug	Subglacial

The procedures for the chemical separation of Sr and rare earth elements (REEs) will be described in detail elsewhere. Measurements of the Eimer and Amend $SrCO_3$ standard over the past eight months range from 0.70807 ± 6 to 0.70818 ± 4 with a mean value for eight samples of 0.70815 ± 4 (R. J. P. and R.K.O'N., unpublished data).

$^{87}Sr/^{86}Sr$ ratios have been determined precisely for two samples from the first day of the present Heimaey eruption, VE-111 and VE-112 (Tables 1 and 2). These two measurements, 0.70329 ± 7 and 0.70330 ± 6 respectively, are in excellent agreement. They may be compared with measurements for samples I-42 and I-43 from Surtsey and earlier volcanic products on Heimaey (VE-67, 72 and 93) (Table 2). There are no statistical differences between the Sr isotope ratios, and it is reasonable to assume that the isotopic composition of the source regions of these magmas has remained constant through late glacial and postglacial times.

Table 2 Strontium Isotope Results

Sample	Locality	$^{87}Sr/^{86}Sr$*
VE-111 (lava)	Heimaey eruption (January 23, 1973)	0.70330 ± 6
VE-112 (tephra)	Heimaey eruption (January 23, 1973)	0.70329 ± 7
I-42 (lava)	Surtsey eruption (1966)	0.70334 ± 4
I-43 (lava)	Surtsey eruption (1964)	0.70330 ± 6
VE-67 (lava)	Helgafell, Heimaey	0.70325 ± 6
VE-72 (volcanic plug)	Háin, Heimaey	0.70323 ± 6
VE-93 (volcanic plug)	Dalfjall, Heimaey	0.70330 ± 5

* Normalized to $^{86}Sr/^{88}Sr = 0.1194$, see text.

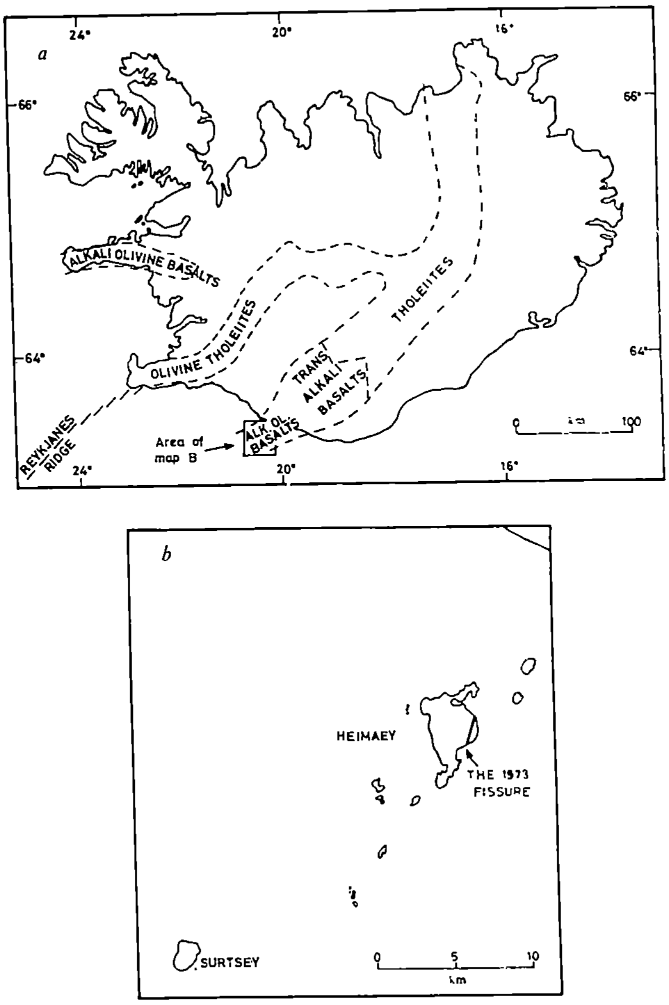

Fig. 1 *a*, Map showing the neovolcanic zones of Iceland, and the areal distribution of petrological basalt types (after Jakobsson[3]). *b*, Sketch map of the Vestmannaeyjar archipelago.

Table 3 Rare Earth Element Concentrations (p.p.m.)

Element	VE-112	VE-67	I-42
Ce	80.58	35.66	22.09
Nd	45.92	22.48	15.27
Sm	11.51	5.88	4.21
Eu	3.24	1.87	1.42
Gd	10.70	5.92	4.64
Dy	10.56	5.55	4.36
Er	6.05	3.21	2.32
Yb	5.39	2.70	1.80

Eight rare earth elements have been measured in each of three rocks, VE-112 and 67, and I-42 (Table 3, Fig. 2). The chondrite-normalized REE distribution pattern in each of these rocks is fractionated with the light REEs showing varying degrees of enrichment by comparison with the heavy REEs. These patterns are broadly similar to those of alkali olivine basalts from other oceanic areas[4], but contrast with those of olivine tholeiite and tholeiite from the mainland (ref. 7 and R. K. O'N. and K. Grönvold, unpublished) of Iceland which do not show marked light REE enrichment and have overall lower abundances. These latter basalts do not on the whole show the characteristic light REE depletion of the dredged ocean ridge tholeiites[7].

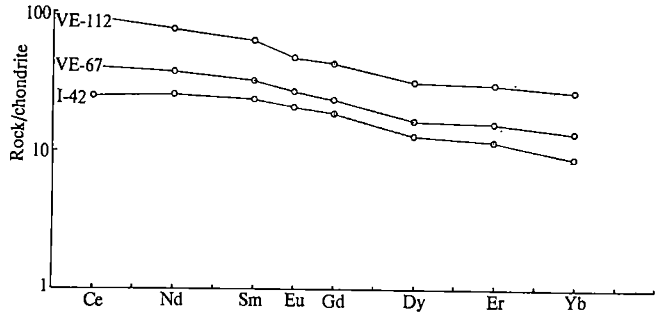

Fig. 2 Chondrite normalized rare earth distributions for a sample of the present eruption on Heimaey (VE-112), a lava sample from a 1966 eruption of Surtsey (I-42), and a sample from Helgafell, Heimaey (VE-67).

The ^{87}Sr/^{86}Sr ratios of the samples analysed here are statistically indistinguishable from the mean of 0.70328 for ten postglacial Icelandic basalts ranging in composition from olivine tholeiite to alkali-olivine basalt (R. K. O'N. and K. Grönvold, unpublished). It may, therefore, be reasonable to assume a common (upper mantle) source region for these basalts which is essentially chemically homogeneous. This would place important constraints on the interpretation of the trace element data.

Recently one of us[3] has suggested, on the basis of seismic determinations of the depth[6] to layer 4, that the alkali basalts in Iceland are generated at greater depths and consequently higher pressures than the tholeiites and the olivine tholeiites, and may therefore represent different degrees of melting of similar material. If the degree of partial melting in the source region has essentially controlled the trace element composition of the various basalt types, then semiquantitative estimates of the relative amounts of melting can be made on the basis of K contents alone. If the olivine tholeiites are generated by $\sim 30\%$ partial melting then the alkali basalts correspond to a factor of five to eight times less melting, that is, 4 to 6% partial melting. The productivity per unit area on surface in the postglacial alkali regions is about five to six times less than in the tholeiite regions. If these estimates are reasonable, the fractionated REE distribution patterns of the alkali basalts (Fig. 2), compared with the relatively unfractionated patterns of the tholeiites, are broadly predictable (R. K. O'N. and K. Grönvold, unpublished) from solid–liquid partition coefficients[8] for a source region composed of olivine + orthopyroxene + clinopyroxene + spinel, or the same with plagioclase instead of spinel. This is because the REE distribution patterns in the case of small amounts of melting will be strongly affected by crystal/liquid partition coefficients for cpx and opx, whereas in the case of more extensive melting olivine will become the major residual phase in equilibrium with the liquid. The absence of a significant Eu anomaly in these basalts favours generation at moderate pressures from a spinel bearing source, or from a plagioclase bearing source at comparatively high f_{O_2} (D. F. Weill and M. J. Drake, unpublished).

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Pleistocene River Gravels and the Stonehenge Problem

On the basis of evidence of glaciation in southern England the presence of glacial ice and associated outwash in areas formerly regarded as ice free throughout the Pleistocene period has been suggested[1–3]. In this account I examine the evidence in Wessex.

Kellaway[1] suggests that during both the "Anglian" and the Wolstonian glaciations, the rivers draining Salisbury Plain (Fig. 1) carried into the Hampshire Basin the outwash of great ice sheets which advanced towards Wessex from the Bristol Channel. He argues that in an "Anglian" glaciation the ice passed across Salisbury Plain and reached Romsey in the Hampshire Basin. Kellaway focuses attention on the distribution of the supposed erratics of the "Anglian" glaciation, and particularly on those erratics of medium-large size. He suggests that some of these have been incorporated into Stonehenge, and into other prehistoric monuments on the Wiltshire Chalk. He also suggests that the plateau gravels of the Hampshire Basin are the disturbed outwash of the "Anglian" ice, and that the valley gravels of the Wylye, Avon and Test can be related to the dissection of the Chalk uplands in the two principal cold periods which followed the "Anglian" glaciation. These gravels must therefore include the outwash of the Wolstonian ice.

I now describe the composition of the Pleistocene gravels of the Wylye, Nadder and Avon, with particular reference to the possible presence of erratic material of small size, and thus the possible relation of the river gravels to outwash of "Anglian" or Wolstonian age. Table 1 shows the composition of twenty-eight samples of gravel from twenty-four sites (mean sample size, 2,210 pebbles) (see Fig. 1).

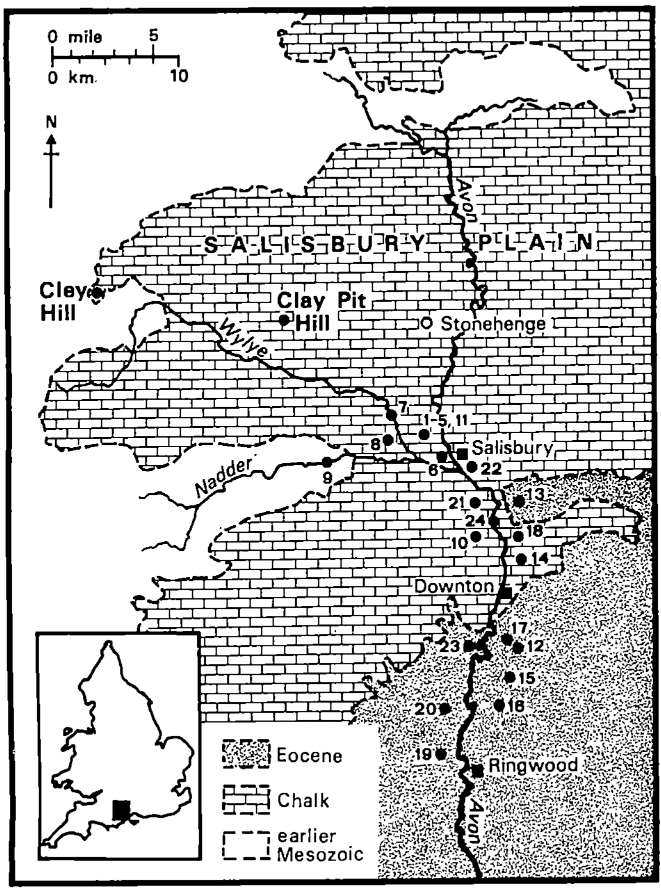

Fig. 1 Salisbury Plain and the Rivers Wylye, Nadder and Avon. Numbers refer to Table 1.

In age, the Pleistocene gravels seem to represent a great part, or perhaps the whole, of the Pleistocene period. The most elevated gravel, at Clearbury Ring (site 10) is at, or close to, the level of the end Tertiary land surface[4]. At a lower level the gravel of site 19 rests on what Sealy[5] terms the Boyn Hill (Hoxnian) terrace of the Avon. Sites 6, 21, 22 and 30 might also be regarded, in terms of elevation and their contained Palaeolithic industries, as of Hoxnian age. Wolstonian outwash could therefore be present at or below this level. Above the Hoxnian level the chronological status of the gravels and terraces is less certain. The terraces of the Salisbury Avon have been discussed by various workers[5-7], who seem to be agreed that a sequence broadly similar to that in the Thames valley is represented. Unfortunately, in the Thames valley, it is not agreed which terrace is contemporary with the "Anglian" glaciation. Both Wooldridge[8] and Sparks[9] have suggested, however, that the outwash of the "Anglian" glaciation at its maximum extent was confluent with the Winter Hill terrace of the Thames. In any case, the "Anglian" glaciation is not likely to relate to a more recent stage than this. On the basis of the broad correlation indicated by Hare[7], between the terraces of the Thames and those of the Salisbury Avon, the position of the Winter Hill and Boyn Hill stages is indicated in Fig. 2.

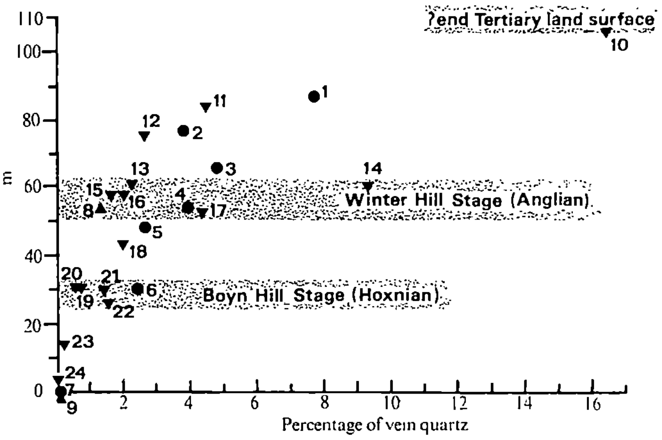

Fig. 2 Frequency of far travelled material (percentage of vein quartz) related to elevation of gravel above the present river (in m). Numbers refer to Table 1.

Several features relative to the composition of the Wessex gravels can be noted. First, the gravels seem to be derived exclusively from outcrops within the existing catchments; they are composed largely, or entirely, of Chalk flint and Upper Greensand chert or sandstone, material supplied from outcrops which are widespread in the basins of the three rivers. Other rocks are present in the gravels in very small amounts. Vein quartz, quartzites, grits and cherts occur. In terms of ultimate origin this material is far travelled; it is also present, however, in nearby Tertiary formations, and could be derived from them. Quartzose gravels, with pebbles of rock of western provenance, have long been known[10] on the western margin of the Hampshire Basin, in the Reading Beds as far north as Downton, and in the Bagshot Beds near Dorchester. Outcrops of similar gravels have been described[11,12] on Salisbury Plain, at Clay Pit Hill and Cley Hill. Thus, the immediate source of the far travelled material in the Pleistocene gravels can also be distinguished within the existing catchments.

Second, Fig. 2 shows that at no stage in the terrace succession in the Wessex area is there evidence of any significant change in the composition of the gravels. In particular, there is no indication of an influx of erratic material in outwash from either "Anglian" or Wolstonian ice. Far travelled material is most

Table 1 Composition (%) of Pleistocene River Gravels

	Wylye–Nadder							Nadder		Avon														
Site No.	1	2	3	4	5†	6*	7	8	9	10	11	12	13*	14	15	16	17	18	19	20	21	22	23	24
Height (m) above river	87	77	66	54	48	30	0	54	0	107	84	76	61	59	58	58	53	44	31	31	30	26	14	3
Flint and Greensand	91.7	96.2	94.8	95.7	96.1	96.1	99.9	98.6	99.9	83.6	95.5	97.2	97.8	90.6	98.2	97.9	95.6	98.0	99.3	99.4	98.6	98.5	99.8	100
Quartz	7.6	3.8	4.6	3.9	2.6	2.4	0.1	1.3	0.1	16.4	4.4	2.6	2.2	9.3	1.6	2.0	4.3	2.0	0.6	0.5	1.4	1.5	0.2	—
Other far travelled	0.7	—	0.6	0.4	1.3	1.5	—	0.1	—	—	0.1	0.2	—	0.1	0.2	0.1	0.1	—	0.1	0.1	—	—	—	—
Size range of sample	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*c*	*b*	*b*	*b*	*c*	*b*	*b*	*c*	*c*	*c*	*b*	*c*	*a*	*b*	*b*	*a*	*b*

* Mean of two samples. † Mean of three samples.
Size range of samples: *a*, 6.3–25.4 mm; *b*, 4.0–12.7 mm; *c*, 3.3–6.3 mm.

frequent at the highest levels above the present rivers and progressively less frequent at lower levels. Assuming a broad correlation between the elevation and age of the gravels, this evidence seems consistent with derivation of the far travelled material from Tertiary formations. This Tertiary material, especially outliers on Salisbury Plain, would be progressively reduced in extent by erosion during the Pleistocene period, and would contribute correspondingly less material to successive Pleistocene gravels.

Finally, the Wessex gravels may be compared with those of the Thames. The influx of glacial outwash into the Thames basin on at least two occasions ("Anglian" and Wolstonian) is not in dispute. Walder[13] shows that the mean content of glacially derived material in the Upper Winter Hill and Rassler terraces near Reading rises to as much as 30.7%. She relates this glacially derived material to the "Northern Drift", a till of the "Anglian" glaciation. Furthermore, much of the glacially derived material in the Thames gravels is of relatively large size. The figures obtained by Walder are based on a minimum sieve aperture of 3/8 inch (approximately 10 mm). Hey[14], using a 16 mm minimum sieve aperture, also reports more than 30% "exotic" material in gravels of the Westland Green stage. In contrast, in the Wessex gravels the far travelled material is almost invariably small. In the coarse fraction (>12.7 mm) of the gravels in Table 1, the mean frequency of far travelled material is 0.14%, representing a range from 0.0% (fifteen sites) to 1.16% (site 19).

The complete lack of glacially derived material in the Pleistocene river gravels of the Wylye, Nadder and Avon seems to show conclusively that the "Anglian" ice never invaded the basins of these rivers, and that the outwash of the Wolstonian ice was never tributary to them. "Anglian" ice cannot therefore be invoked to explain the presence of large and medium sized Welsh rocks, and other rocks of distant origin at Stonehenge, or in other prehistoric monuments on the Wiltshire Chalk. The problem of the introduction of these rocks to this area remains unsolved.

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"Burtle Clay" of Somerset

Kidson and Haynes[1] following Kidson[2,3] claim that marine or estuarine deposits which they call "Burtle Clay" underlie the Burtle Beds of Somerset. The sands and gravels of the Burtle Beds have been described as marine deposits[4] but a glacial origin has recently been ascribed to them[5,6]. It is claimed[1] that the Burtle Clay is of considerable stratigraphical significance, that it proves the occurrence of a widespread marine transgression, destroys the case for the glacial origin of the sands and gravels of the Burtle Beds, and throws doubt on the theory of glacial transport of the "bluestone" erratics incorporated in Neolithic and Bronze age monuments on Salisbury Plain. We find nothing to convince us of the marine origin of the Burtle Beds or to change our opinion about the older glaciations.

The Burtle Clay has not been described from any surface exposure, nor has six-inch mapping of the Burtle Hill area by the Geological Survey revealed any evidence of its presence. One "borehole" 3 foot (1 m) deep at Lilac Farm (Fig. 2 of ref. 1) is shown as having penetrated the Burtle Clay beneath a thin cover of sandy soil, but the material contains no foraminifera or ostracods and the description suggests to us a weathered tufaceous clay. We have not been able to find the precise location of borehole 8F and therefore cannot confirm the presence of tufa or "marl" in the area covered by the six-figure grid reference to Lilac Farm[1]. The "borehole" seems to have been near the junction of peaty Holocene deposits and the sands of the Pleistocene Burtle Beds. Tufaceous clays commonly occur in this kind of situation in Somerset[7-9]. In any event there is no evidence to support a marine transgression.

South of the Poldens the only material which has been described as Burtle Clay is the red mud below the Burtle Beds in borehole 8K at Cutley Farm near Othery (Fig. 2 of ref. 1). Here it is shown as 1 foot (0.3 m) thick and presumably rests on red Keuper Marl which is the bedrock in this area. It seems to us most likely that this material is the weathered top of the Keuper Marl churned up by a drill bit or auger and contaminated by microfossils derived from the overlying Burtle Beds.

North of the Poldens the only boreholes showing any appreciable thickness of Burtle Clay are the shallow holes 8D, 8E and 8H (ref. 1). Two of these, 8D and 8H, passed through the Burtle Beds (sand and gravel) before encountering the problematical Burtle Clay which is shown as 7 to 12 foot (2.1 to 3.7 m) thick. No detailed comparison between the microfauna of the Burtle Beds and the Burtle Clay in the boreholes in the Burtle Hill area has been given by Kidson and Haynes[1], but most of the Quaternary foraminifera recorded[1] from the Burtle Clay are comparable with species which we have found in the Burtle Beds at Westonzoyland. In borehole 8E, where there is no overlying Quaternary sand and gravel, the Burtle Clay is apparently devoid of Quaternary foraminifera (Fig. 3 of ref. 1). The relatively low foraminifera counts in the Burtle Clay of 8D and 8H also suggest contamination. Yet neither the drilling equipment, the method of sampling, percentage recovery in the cores or samples, nor the possibility of contamination and remoulding of the material are mentioned. This information is important because remoulding and contamination of the Lias clay could give rise to samples such as those described as "doughy clay" (Fig. 1.6 of ref. 3). Alternatively this material could be till or head.

We have tried unsuccessfully to prove (by means of augering) the Burtle Clay shown in borehole 8E. Because of the inadequacy of the grid reference, we have been unable to locate the site of the three boreholes at Catcott Burtle. As two of the boreholes are shown in Fig. 1 of ref. 1 to be 500 yards (457 m) apart we presume there is a mistake in the grid reference.

The Burtle Clay of the Burtle Hill area is probably remoulded Lower Lias clay contaminated with microfossils or ground up shell debris (Fig. 1.6 of ref. 3), some having been carried by water draining from the overlying Burtle Beds in the boreholes. This, we think, may explain why the Burtle Clay seems to contain no Quaternary microfossils where there are no overlying Burtle Beds. The fact that no "Liassic" foraminifera occur at the top of the Burtle Clay in boreholes 8D and 8H suggests that they have been removed by weathering before the deposition of the Burtle Beds.

We have had difficulty in understanding the article by Kidson and Haynes[1]. The key to Fig. 2 is incomplete, but we assume from the context that the broken horizontal shading on the borehole sections indicates *in situ* Jurassic rocks. Then the presence of the Bucklandi Zone at the sub-drift surface in borehole 8G, and of "Middle and Upper Lias foraminifera" at the bottom of 8E, implies that a major fault, previously unknown, passes between two holes that are so close together that a single grid reference is given for both. As the Lower and Middle Lias in this part of Somerset is more than 1,400 foot (427 m) thick[10] and the top of the Bucklandi Zone is about 300 foot (91 m) above the base of the Lias, it follows that the fault would have a throw of about 1,100 foot (335 m). This is roughly twice the displacement of any comparable post-Liassic fault known in the central Somerset basin and is not likely to have missed detection either by geological or geophysical surveys.

Of course the clays beneath the Burtle Beds at Burtle Hill might be weathered till or head, but it seems more likely to us that the samples examined by Kidson and Haynes[1] are remoulded Lower Lias clay. Whatever the explanation, it is clear that the presence in borehole 8E of clay with a Liassic microfauna but devoid of Quaternary forms cannot possibly be regarded as evidence for a Quaternary marine transgression. We also consider that the use of the term "Liassic" as applied by Kidson and Haynes[1] to the foraminifera listed under this title in Fig. 3 is misleading. Many of the genera have ranges extending from Permo-Triassic to Recent and one (*Ammodiscus*), Silurian to Recent[11]. There may be Jurassic species of some of these genera in the Burtle Beds at Burtle Hill, and some of these could have become incorporated in the samples of Burtle Clay. But the genus *Planularia*, listed as "Liassic"[1], is usually considered to be Miocene–Recent[11] and we fail to see how it can be maintained that the only "time periods" represented by the microfossils of the Burtle Clay are exclusively "Lias" or Quaternary. Indeed, we deprecate the use of the term "Lias" in this context, as the total thickness of the Lower, Middle and Upper Lias before erosion may well have been of the order of 1,700 to 2,000 foot (518 to 610 m). Of this, only some 300 foot (91 m) of Lower Lias remains beneath the Burtle Beds at Burtle Hill. Any species of foraminifera of post-Sinemurian age, whether Jurassic, Cretaceous or Tertiary, found in the Burtle Clay could have been derived from the Burtle Beds but could not have been derived from the underlying Lower Lias.

We consider it regrettable that the term "Burtle Clay", based on such unsatisfactory evidence, should have been submitted for acceptance, particularly when attempts are being made through the Geological Society of London to improve standards of stratigraphical definition.

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Tidal Patterns and Energy Balance in the Gulf of California

Roden and Groves[1] wrote: "The oceanographic aspects of the Gulf of California, one of the least known marginal seas of the North Pacific, are both interesting and complex". Tidal processes certainly justify the statement. With a reported spring range of 10 m as well as currents exceeding 6 knots (ref. 2) the tides in the Gulf of California are among the most spectacular and dangerous in the world. Nevertheless, because of the rarity and inadequacy of observations, no consistent picture could be drawn until recently. Many quantitative studies of this area must thus be regarded as provisional; there is, for example, an eight-fold range in estimates of energy dissipation for the semidiurnal lunar tide, namely, Heiskanen[3,4]: 2.5×10^{17} erg s^{-1}; Miller[5]: 4×10^{17} erg s^{-1}; Hendershott[6]: 1 to 2×10^{17} erg s^{-1}; and my proposed values: 4.35×10^{16} erg s^{-1} for M_2 alone, 4.65×10^{16} erg s^{-1} for all semidiurnal lunar constituents and 4.85×10^{16} erg s^{-1} for all lunar constituents.

I have recently recorded tidal heights and temperature by means of self-contained pressure recorders laid on the bottom

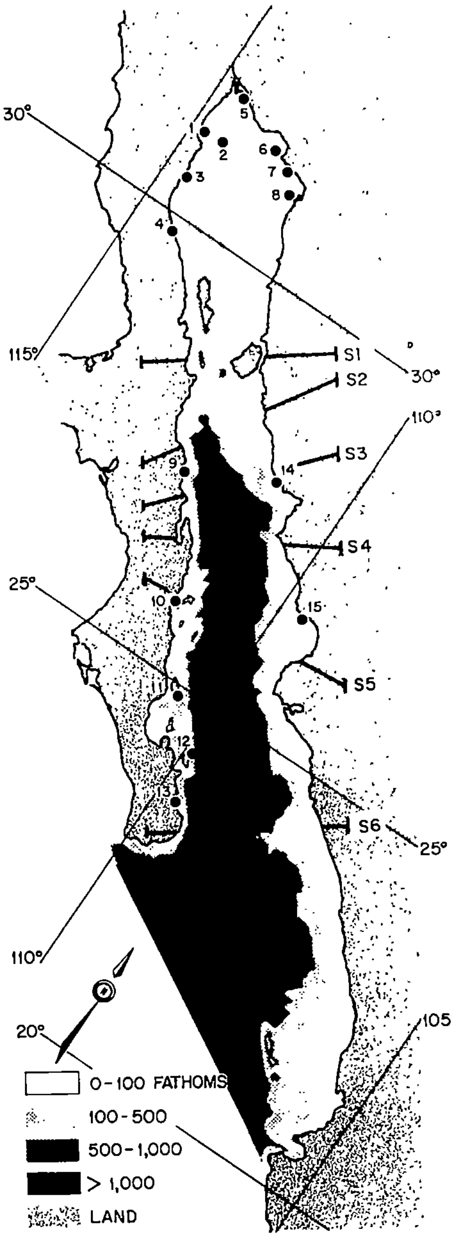

Fig. 1 Depth contours, identification and position of stations (1, 2 . . . 15) and identification of sections for which various estimates are tabulated (S_1, S_2...S_6) for the Gulf of California.

at fifteen open coast or island locations at positions well removed from local influences (Fig. 1). The records cover 10 to 60 days, and with appropriate correction by convolution of the temperature trace with the instrumental response, are accurate to within about 1 cm.

The resulting time series have been related to tidal driving forces through frequency dependent diurnal and semidiurnal transfer functions[7]. Amplitudes and phases for the chief diurnal and semidiurnal constituents K_1O_1 and M_2, S_2 are listed in Table 1.

From the relatively dense data arrays resulting, cotidal and corange tide patterns can be readily sketched with remarkable certainty. When complemented with accurate—and available—coastal and bottom contour maps[8] one may calculate accurately the tidal prism P_n or the volume of water above or below mean sea level to the head of a section n across the Gulf, the transport T_n, or rate of water inflow across section n, the potential energy distribution, E^p, the gravitational energy flux, E^g, or the energy contributed directly over the Gulf by the gravitational forces of Sun and Moon as they enhance or hinder the flow of water from the adjacent Pacific Ocean, and the advective or oceanic energy flux E^0 which represents the energy penetrating from the Pacific Ocean into the Gulf. With a smaller yet useful accuracy one may also calculate the average velocity v_n across a section n, the kinetic energy E^k associated with the moving water, and the ratio R of the amplitude of the tidal wave returning toward the ocean after reflexion in the Gulf to the amplitude of the entering wave. When gravitational driving is small compared to oceanic driving, R is called the reflexion coefficient. By insuring smooth variation of these quantities along the Gulf, yet preserving the fit of observations, I have obtained optimum tidal patterns.

Cotidal and corange maps for the principal semidiurnal constituent M_2 (Fig. 2) illustrate the behaviour of the semidiurnal tide. The diurnal tide is almost in phase (within 6°, or 24 min, Table 1) over the entire Gulf. It is, therefore, almost fully described by its corange lines (Fig. 3). The evidence is however, strongly in favour of the nearly axial orientation of

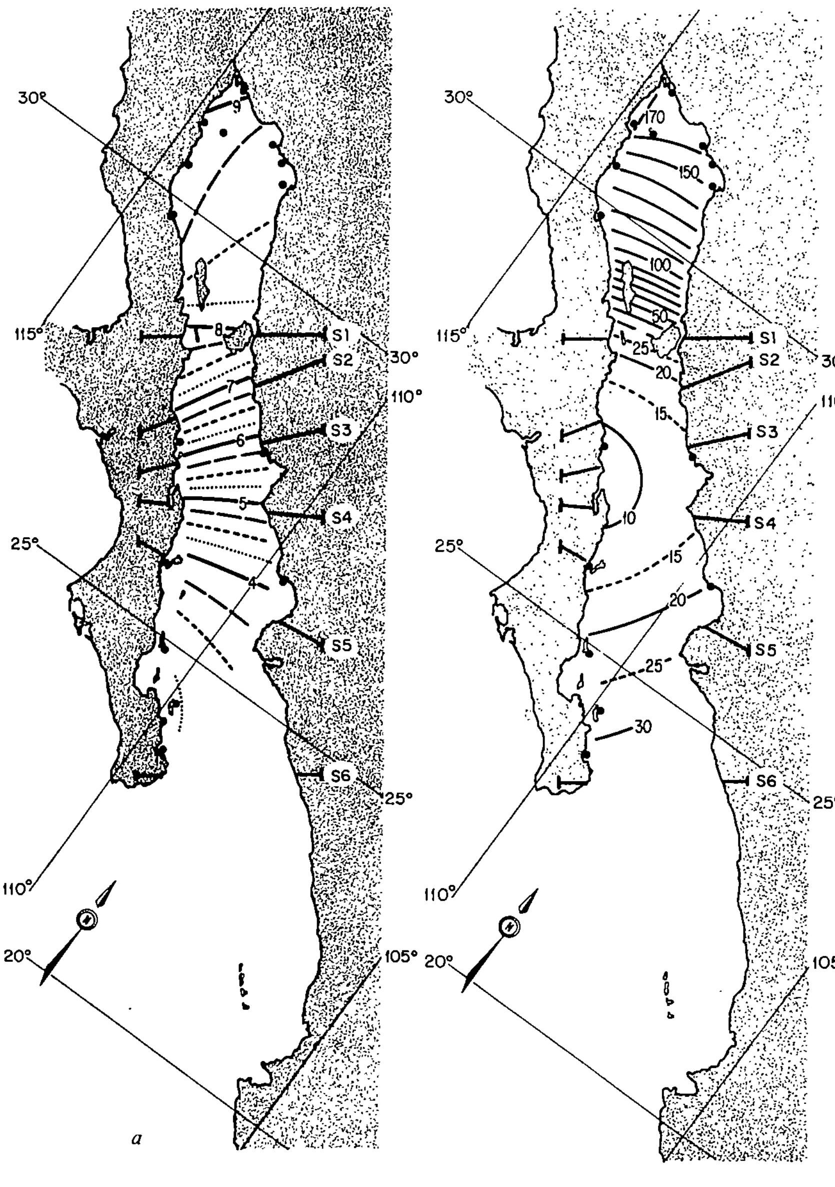

Fig. 2 Cotidal (*a*) and corange (*b*) maps for semidiurnal constituents M_2. *a*, Labels on cotidal lines refer to lag in lunar h behind equilibrium tide at Greenwich; *b*, labels on corange lines are in cm.

the limited cotidal pattern shown in dotted lines at intervals of 4 min (Table 1, K_1 and O_1).

The difference between semidiurnal and diurnal tides is striking. The semidiurnal tide enters the Gulf with a moderate amplitude (30 cm for M_2), slows down inwards, decreasing to one-third its initial amplitude near the middle of the Gulf. It then accelerates while its amplitude rises to fifteen times its amplitude in the middle of the Gulf at the head (165 cm for M_2). There it seems to be absorbed by the coastline, suggesting large dissipation as also indicated by the shift into virtual existence of an amphidromic region near the middle of the Gulf as pointed out by Hendershott[6]. By contrast, the amplitude of the diurnal tide increases very slowly and monotonically to less than twice its amplitude at the mouth. Its nearly uniform phase suggests that it is like a standing oscillation, and that most energy entering the Gulf does leave again.

A summary of the calculations mentioned earlier is given in Tables 2 and 3. The complete estimates used, for convenience, are elements bounded by the coast and by cotidal lines for M_2 at intervals of 15 min (Fig. 2). They were obtained as follows:

$$P_n = \underset{n}{\Sigma} h_n \Delta A_n$$

where $\underset{n}{\Sigma}$ indicates a summation from 1 to n, h_n is the average tidal elevation over the element n with area ΔA_n

$$T_n = \mathrm{d}P_n/\mathrm{d}t$$
$$v_n = T_n/S_n$$

where S_n refers to the area of the cross-section of the Gulf

$$E_n^g = \underset{n}{\Sigma} \langle T_n \nabla G_n \Delta l_n \rangle$$

where ∇G_n is the horizontal gradient of the gravitational potential, Δl_n is the mean tidal motion within an element n and the symbol $\langle\ \rangle$ indicates time averaging

$$E_n^0 = T_n h_n \cos \Phi_n$$

where Φ_n is the phase difference between transport and tidal elevation.

The time-averaged dissipation of energy D is the sum of the gravitational and oceanic energy fluxes, and the total energy stored in the Gulf is the sum of the potential and kinetic energy with

$$D_n = E_n^g + E_n^0$$
$$E^p = \tfrac{1}{2} \Sigma_n \rho g h_n^2 \Delta A_n$$
$$E^k = \tfrac{1}{2} \Sigma_n \rho \Delta A_n S_n V_n^2$$

where ρ is the specific gravity of seawater and g is the acceleration of gravity.

Table 1 Amplitudes and Phases (with Respect to Equilibrium Tide at Greenwich) of the Chief Diurnal Constituents K_1, O_1, and of the Chief Semidiurnal Constituents M_2, S_2

Station identification	K_1 Amplitude (cm)	Phase (°)	O_1 Amplitude (cm)	Phase (°)	M_2 Amplitude (cm)	Phase (°)	S_2 Amplitude (cm)	Phase (°)
1	43.4	188	29.0	176	173.2	265	94.4	273
2	45.0	186	27.6	176	163.4	263	91.8	270
3	42.8	184	28.7	178	132.1	267	76.3	278
4	42.5	184	28.6	175	115.0	265	37.8	261
5	49.4	184	30.8	171	161.9	272	114.8	268
6	46.1	180	30.4	172	161.8	259	94.9	265
7	46.1	179	30.1	171	154.6	257	89.4	263
8	45.8	179	30.0	172	150.9	257	68.7	274
9	31.8	184	21.9	171	7.2	193	6.0	144
10	30.1	184	20.1	171	14.5	115	13.9	118
11	28.6	184	20.0	170	24.2	101	19.4	108
12	27.5	185	19.2	172	27.5	97	20.8	107
13	26.71	182	18.7	168	30.7	98	22.5	104
14	30.6	183	21.6	169	13.0	159	10.4	139
15	28.0	183	20.4	166	20.2	123	16.9	121

Table 2 Tidal Prism, Transport, Space-Averaged Peak Velocity, Energy Fluxes, Stored Energy and Reflexion Coefficients for M_2 Tide at Six Representative Sections

	Prism				Energy fluxes				
Section	Volume (10^{16} cm^3) P_n	Phase (°)	Transport (10^{12} cm^3 s^{-1}) T_n	Velocity (cm s^{-1}) V_n	Oceanic (10^{16} erg s^{-1}) E_n^0	Gravitational (10^{16} erg s^{-1}) E_n^g	Dissipation (10^{16} erg s^{-1}) D_n	Stored energy (10^{21} erg) $E_n^P + E_n^K$	Reflexion coefficient R_n
S_1	5.53	258	7.77	38.8	3.66	−0.07	3.59	3.71	0.87*
S_2	5.74	257	8.06	23.0	4.08	−0.13	3.95	3.75	0.77
S_3	5.78	256	8.12	8.7	4.14	−0.17	3.97	3.75	0.72
S_4	5.77	255	8.11	5.6	4.26	−0.19	4.08	3.76	0.73
S_5	5.59	252	7.86	5.1	4.43	−0.22	4.21	3.78	0.75*
S_6					4.70	−0.35	4.35	3.80	

* Accuracy reduced because of small number of observations.

Table 3 Data* for K_1 Tides at Section S_6 (Oceanic Boundary of Gulf)

S_6	4.21	184	3.1	1.5	0.10	0.08	0.18	0.87	0.95

* Same column headings as for Table 2.

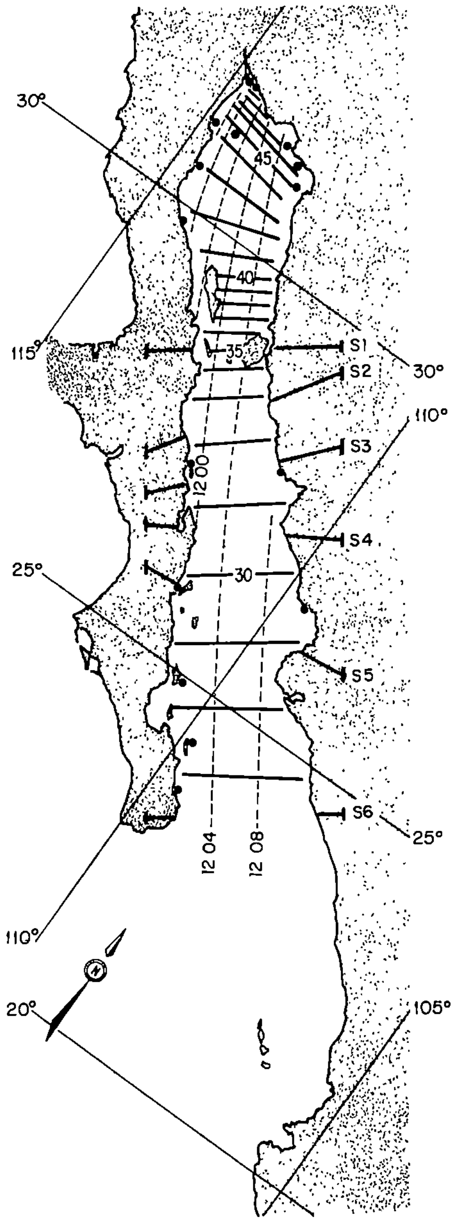

Fig. 3 Corange map for diurnal constituent K_1. Labels on corange lines are in cm. Cotidal lines (- - -) are labelled in h and min behind equilibrium tide at Greenwich.

Owing to fanning out of the semidiurnal patterns south-east of section S5 and to the limited coverage of the observations, detailed calculations were not performed in this area for the semidiurnal tide. Nevertheless, semidiurnal dissipation can be extrapolated with confidence for the whole Gulf, assumed to be bounded by section S6.

At semidiurnal frequency the area of the Gulf to the north of section S4 may be regarded as a tuned quarter wave oscillator because the tide covers this space exactly one quarter of my tidal cycle. The southern half acts as an almost non-dissipative element connecting the northern half to the ocean (zero impedance).

A classical method of appraising the degree of damping—or dissipativeness—of a physical system is to estimate its resonant factor, Q. The latter is 2π times the number N of cycles required to dissipate an amount of energy equal to that stored in the system

$$Q = 2\pi N = 2\pi \text{ stored energy/dissipation per cycle}$$

For the northern Gulf, which resonates at semidiurnal lunar frequency, $Q_{M2} = 12.5$. This value rises slightly, to 13.2, for the entire Gulf. Hence, the tidal energy stored in the Gulf is dissipated in roughly two tidal cycles for M_2.

The natural resonance frequency (F_R) of the whole Gulf is about 1.8 cycles day^{-1} whether derived from wave propagation time with reflexion very near the most distant north-west shore, or from the phase relationships between waves entering and leaving. In the second case the effect of gravitational driving, which is negative for the semidiurnal tide and which amounts to about 5% of oceanic driving, has been neglected (Tables 2 and 3). The consistency of the results provided by the two methods confirms that most dissipation occurs in the shoals in the north-west of the Gulf as already indicated by Table 2.

The observed lag of S_2 with respect to M_2 (about 6) is consistent with the values of F_R and Q_{M2} (ref. 9) if their oceanic phases are the same, a situation that seems plausible but cannot be fully established with existing data.

Calculations related to the much simpler diurnal tide are summarized in Table 3, for K_1. Dissipation is smaller by comparison with stored energy than for M_2 ($Q_{K1} = 30$); gravitational driving is positive and constitutes nearly as much as oceanic driving.

Table 4 Time Average Dissipation for Whole Gulf (10^{16} erg s^{-1})

	Lunar	Solar
Diurnal	0.2	0.07
Semidiurnal	4.95	1.65
Total	5.15	1.72

Estimates of total dissipation, based on eleven constituents in each species, are given in Table 4, together with diurnal and semidiurnal, lunar and solar contributions. The total time-averaged lunar dissipation is 4.85×10^{16} erg s^{-1}, of which 4.35×10^{16} erg s^{-1}, or 90%, is associated with M_2. The uncertainty in these figures, 10 to 15%, is chiefly a consequence of gaps in the observational coverage. One advantage of the present method is that it derives the required barotropic, or average, velocity field from precisely known time rates of water volume change. Thus baroclinic—or turbulent—motion is systematically eliminated, a requirement beyond the scope of available meters. The electromagnetic approach to estimation of barotropic motion, now almost operational and based on measurements on the seafloor of the electric field generated by water moving through the Earth's magnetic field, may soon confirm these estimates[10].

Because tidal dissipation must have played an important role in the evolution of the Earth–Moon system[11], many attempts have been made to justify the agreement between estimates of tidal dissipation based directly on suspected dissipative mechanisms with those derived astronomically. With appreciable contribution from solid Earth tides dismissed on the grounds of high values for the resonant Q factor of the lowest Earth modes[11], attention is directed to the ocean. Important dissipation on the deep ocean floor, in spite of its dominant extent, is unlikely because bottom velocities are very low there, while friction in the case of a turbulent boundary layer follows a square law dependence on velocity. Thus, most tidal dissipation has been assumed to result from bottom friction in shallow and certain marginal seas with high tidal activity. Estimates of the dissipation in these areas have been attempted for more than half a century in spite of frustratingly inadequate data.

Present estimates for the Gulf of California are considerably lower than any previous ones. Such a situation arose earlier with respect to the Bering Sea[4,12]. Should such a trend continue to be reflected in future investigations, dissipation mechanisms other than bottom friction of tidal currents may have to be ascribed an important role. If so, energy scattering into internal modes, followed by degradation into fluctuations of higher frequencies[13], is the most probable explanation.

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Explosive Interaction of Liquefied Natural Gas and Organic Liquids

WHEN liquefied natural gas (LNG) is spilled onto water, a violent explosion may occur[1], caused by the sudden release of energy stored in a superheated, metastable LNG phase. It is therefore termed a superheat-limit explosion (SLE)[2,3]. A SLE does not contain flames, but is very noisy. According to one theoretical estimate[4], the explosion which can occur when LNG is spilled onto water is equivalent to 1/50 to 1/17 the explosion of a similar mass of TNT. Because the LNG would be spread over a much larger area, however, the force per unit area from such an explosion would apparently not be large.

SLE on water has been shown (Enger *et al.*, cited in ref. 3) to occur only when the LNG contains abnormally high amounts of heavier hydrocarbons ($\geqslant$50 mol %); such a situation would not normally occur in practice and therefore SLE on water is not a point of great concern in the LNG industry. But in view of the very large number of LNG transport schemes now under consideration, which might involve the use of as many as 100 LNG tankers, it seems prudent to gain the best possible knowledge of how and why such explosions can occur.

We discuss here explosions which occur when LNG is poured onto various organic liquids. Occurrences of SLE on *n*-pentane have been mentioned before[3], but for other organic liquids there is no published information. By sharp contrast with water, where a high concentration of heavy hydrocarbons in LNG is needed to cause SLE, explosions on some organic compounds occur even if LNG has its usual composition, less than 10% heavy components and more than 90% methane. The nature of the compounds which cause the LNG explosions gives useful information on the SLE mechanism.

The experimental arrangement was assembled in a well ventilated hood. An organic liquid (15 ml) to be tested was introduced in a 30 ml 'Pyrex' beaker at room temperature. LNG (2.5 ml), taken from a Dewar with a pre-cooled plastic measuring spoon, was then poured into the liquid. Liquid spill-over, caused by the explosion, was collected in a water-filled tray placed below the beaker.

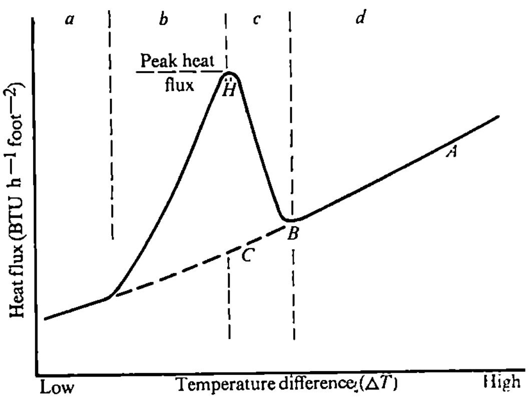

Fig. 1 Boiling heat transfer mechanism[2,3]. Heat transfer modes: *a*, Convection and conduction; *b*, nucleate boiling; *c*, superheat region; *d*, film boiling. *H* represents the region of peak heat flux.

The gas from which LNG was prepared had the following composition (mol%): N_2 (1.5), CO_2 (0.2), CH_4 (90.1), C_2H_6 (5.4), C_3H_8 (2.1), *iso*-C_4H_{10} (0.2), *n*-C_4H_{10} (0.4), and *iso*-C_5H_{12} (0.1). Phillips CP grade methane and ethane were used to prepare liquid methane and liquid ethane.

With LNG, the liquids which caused reproducible explosions were: *n*-pentane, *iso*-pentane, *n*-hexane, 2.2-dimethylbutane, 3-methylpentane, 2,3-dimethylbutane, 2-methylpentane, *n*-heptane and *n*-octane. With this group of liquids, the induction period was quite short; explosions occurred 2–3 s after pouring the LNG. Methanol, acetone and methylethyl ketone also caused reproducible explosions, but here the induction period was appreciable (about 5 s, sometimes longer). Explosions on ethanol and isopropyl alcohol were not reproducible, and occurred only in 5–10% of trials. No explosions were observed on *sec*-butyl-alcohol, *n*-nonane, *n*-decane or benzene.

By contrast with LNG, explosions with liquid methane were observed on *iso*-pentane, but on no other liquids.

Liquid ethane, poured into various liquids, simply vaporized instantaneously. With liquid ethane, SLE-type explosions were not observed on any of the liquids tested under the present experimental conditions.

Fig. 1 has been used before to discuss SLE on LNG-water systems[2,3]. Here the rate of heat transfer from liquid to LNG is plotted against the temperature difference between cold LNG and the comparatively warm liquid below it. Initially ΔT is very high and the system is in the film boiling range. In this range, two liquid phases are separated by a thin vapour layer, and the heat transfer rate is quite low. As time elapses, ΔT decreases and the system approaches the superheat region (Fig. 1*B*–*C*). At this stage, the film collapses, two liquid phases come into contact and the rate of heat transfer suddenly increases. In certain conditions, this rapid heat transfer leads to the formation of a metastable, superheated LNG phase. Subsequently, stored energy is released explosively when the tensile strength of LNG is exceeded.

An important condition for the formation of the metastable state is that when the system is in the superheat region, the liquid below the LNG must remain free of any solid matter resulting from freezing. Otherwise, the solid provides nuclei for rapid boiling, and no superheating occurs. Freezing may result if the liquid has a high melting point[5], or if heat transfer from the bulk phase to the LNG interface is too slow to compensate for heat loss there. Thus in a homologous series the higher members are more likely to freeze at the interface. On this basis, the occurrence of

SLE on C_5, C_6, C_7 and C_8 hydrocarbons but not on C_9 or C_{10} hydrocarbons is explained. Similarly, the occurrence of SLE on methanol but not on butanol is in accord with the expected trend. The somewhat longer induction period in methanol and acetone, by comparison with hydrocarbons, may arise because the solubility of LNG vapour in the former is low. This prolongs the period of film boiling because of poor heat transfer.

With liquid methane, the film boiling range is much longer than with LNG. Here it seems that most liquids freeze before, or while, superheating occurs. Only *iso*-pentane, which has the lowest melting point (−160° C) among the compounds investigated, remains liquid at the interface and leads to explosions.

With liquid ethane, instead of explosion, only rapid vaporization occurs. This is true even if the liquid ethane is cooled to −160° C before pouring so as to provide an initial ΔT which is comparable with that encountered in the experiments with LNG. It seems that, because of the high boiling point of ethane (−88° C), the system approaches the superheat region very rapidly (<1 s). When this happens, heat transfer from the bulk liquid phase may not be rapid enough to prevent freezing at the interface. It is hence not surprising that the metastable state is not formed in liquid ethane under the experimental conditions described here.

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Plumbosolvency of Waters

SOME cases of lead poisoning from domestic cold water supplies have been described[1,2] and in a study of the lead content of water in Glasgow, where many older buildings have lead plumbing, a significant correlation was found between the length of lead pipe, the water lead content and blood lead of individuals resident in properties with lead plumbing[3]. In the following experiments a sample of old domestic lead piping was obtained to examine the effects of various factors on the relative plumbosolvencies of waters from various towns.

The lead piping used in the experiment was a typical piece of lead pipe (internal diameter 0.5 inch) which had previously been used for about 50 yr. Measurements of lead content were made after 1 h of contact of water with the pipe. Plumbosolvency is defined as the rate of solution of lead and is expressed as μg Pb l^{-1} h^{-1}. The lead content of the water was determined on a Shandon-Southern A1660 polarograph. Calcium and magnesium levels were measured by atomic absorption spectrophotometry.

The pipe was longitudinally sectioned and its interior surface examined microscopically. It was found to be deeply fissured, with deposits of grey lead salts.

To investigate the effects of *p*H HCl and NaOH solutions were used, covering a range from 3–11. It was found that the plumbosolvency of the water increased considerably outside the *p*H range 6 to 8 (Fig. 1). Similar results were found for the water from the towns. A natural peaty water was obtained from a moorland pool in the Kilsyth Hills. This water had a *p*H of 5 and, although as hard as the water from Edinburgh, it had a very much higher plumbosolvency.

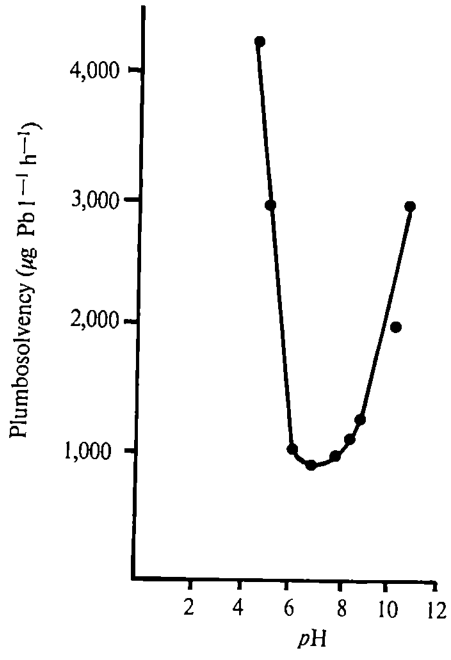

Fig. 1 Effect of *p*H on the plumbosolvency of distilled water. In this experiment the water was allowed to stand in the pipe section for 1 h with *p*H (adjusted with HCl or NaOH) being measured before and after this period. Temperature was maintained at 20° C.

There was a marked effect of temperature on the solution of lead from the pipes. As shown in Fig. 2 there is an exponential increase (plumbosolvency=101 exp(0.01T)) in the plumbosolvency of the distilled water up to a maximum temperature examined (60° C).

Table 1 shows the measured parameters for the tap water from the various towns. The *p*Hs lay between 6.2–6.6, similar to the *p*H of distilled water. The peaty burn water had a much lower *p*H of 5.1. Water hardness, assessed by the levels of calcium and magnesium in the water, was found to be in the order: distilled water< Glasgow<Edinburgh<Moorland<Paisley<London.

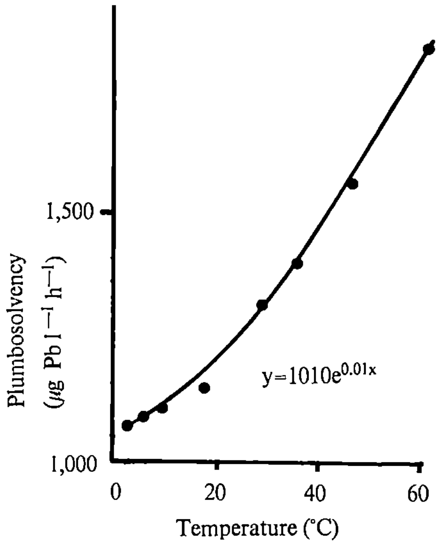

Fig. 2 Effect of temperature on plumbosolvency of distilled water. The temperature was maintained at the given value in a cold room if below room temperature or in a thermostatically controlled oven. *p*H remained constant at 6.2.

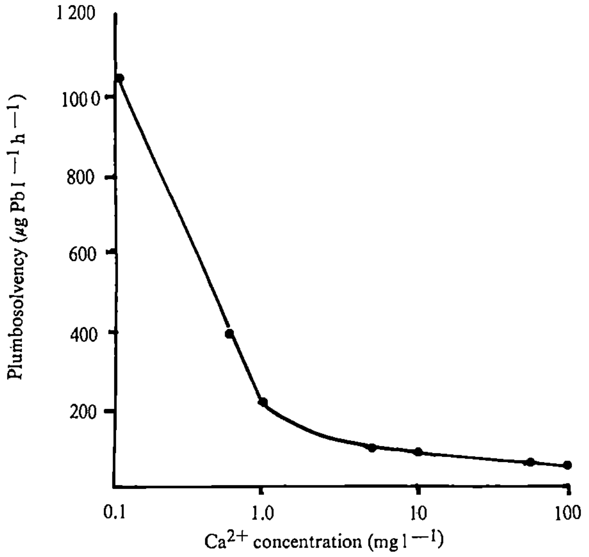

Fig. 3 Plumbosolvencies of synthetically hard water. Distilled water was hardened with calcium chloride to the concentrations shown. The pipes were kept at 20° C and in contact with water for 1 h.

In view of this, the plumbosolvency of synthetic hard water was examined. It was found that the relationship was a power curve where

$$\text{Plumbosolvency} = 306.3(\mathrm{Ca}^{2+})^{-0.4}$$

It is in the region of normal water hardness, 0.1 mg $l^{-1} > Ca^{2+} > 10$ mg l^{-1}, that there is a very rapid increase in plumbosolvency.

The effect of duration of flushing of water was determined in property supplied with water from lead piping. The length of lead supply pipe was 31 foot from the cast iron main. Lead levels in the water started at 272 μg l^{-1} but rapidly fell to normal reaching an equilibrium level of 31 μg l^{-1} after running the water for only 2 min at 12 l min^{-1}.

Table 1 Lead Corrosion Rates and Analytical Data for a Number of Waters

Water	Hardness Calcium (mg l^{-1})	Hardness Magnesium (mg l^{-1})	pH	Plumbo-solvency (μg l^{-1} h^{-1})
Distilled	0	0	6.2	1,121
Glasgow	0.33	0.1	6.5	930
Paisley (Stanley filter)	1.15	0.24	6.6	462
Edinburgh	1.0	0.21	6.6	515
London	10.6	0.56	6.3	230
Moorland burn (Kilsyth Hills)	1.21	0.25	5.1	860

The use of lead pipes for cold drinking water supplies has been common since the days of ancient Rome and the health hazard has been known since that time. Beattie *et al.*[3] showed that in Glasgow the levels of lead in water were greatly in excess of the WHO's recommended limit of 100 μg l^{-1}. Glasgow has a very soft water supply, derived chiefly from the natural reservoir of Loch Katrine. This reservoir, first suggested in the nineteenth century, raised some doubts as to the possible injurious effects of the increased plumbosolvency of the soft water. The opinions were sought in 1855 of Stephenson and Brunel. Their report stated that "Water of precisely similar quality and having the same power has been hitherto so used in contact with lead without any evil consequences"[4]. The techniques for lead determination at that time were insufficiently sensitive to detect the small, but possibly injurious, quantities of lead in the water.

Paisley has, since the 1930s, been supplied with water which is passed through limestone (calcium carbonate) Stanley filters. In 1960 part of the town was changed to a softer supply from Loch Katrine, thus providing the basis for a controlled study. Our results indicate that the plumbosolvency in those houses supplied from Loch Katrine is at least twice as great as those supplied from the Stanley filtered supply.

An additional safeguard in hard water areas is that a "scale" of calcium salts may form on the inside of the pipe, further reducing the plumbosolvency of the water[5]; in soft water areas corrosion increases the surface area available for lead solution. The other factors affecting plumbosolvency, temperature and *p*H, have little effect on cold water plumbosolvency, unless the water supply is acidified, for example from peaty moorland sources.

The hazard of lead exposure from domestic water supplies, though small, must be of some concern. The water as supplied by all local authorities in Britain is well within the WHO recommended limit of 100 μg l^{-1} at source and indeed also at the tap in most cases. It is only when water is allowed to stand in lead pipes that a more concentrated lead solution forms. Thus it is important that in properties with lead piping, the "stale" or lead-contaminated water should be run away before domestic use; we have shown that 2 min flushing reduces the lead content to safer levels.

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Chemistry of the Conversion of Nitrate Nitrogen to Smoke Products

NITRATE nitrogen has been of interest to us because of its natural occurrence in burley tobaccos and of its use as a tobacco additive. Sodium nitrate added to tobacco is known to reduce particulate matter, nicotine, phenol, and benzo(a)-pyrene in cigarette smoke[1]. It is thought that nitrates form oxygen and nitric oxide during smoking and thereby intercept radicals that would otherwise form polynuclear aromatic hydrocarbons. Nitroalkanes are known to increase in smoke as a result of the addition of alkali nitrate to tobacco and tobacco sheet[2], and catechol concentrations in smoke are known to be lowered if nitrate is added to tobacco[3]. A study which involved the fifth puff of a cigarette only, reports sodium nitrate to raise the levels of nitrogen oxides, acetaldehyde, nitriles and acrolein in the smoke[4] (Table 1).

Nitrates labelled with ^{15}N were used to follow the incorporation of nitrate nitrogen into smoke products. Cigarettes (1.14 g, 85 mm, non-filtered, 21 s porosity citrate paper) were hand-made from Kentucky Reference 1R1 tobacco which had been sprayed with aqueous solutions of the appropriate ^{15}N-labelled nitrate [$Ca(^{15}NO_3)_2$, 95 atom %; K and Na $^{15}NO_3$, 99 atom %].

Total nitrogen and nitrate nitrogen analyses showed the amounts of nitrate (as N) added to be 1.8, 1.5 and 1.8% for the calcium, potassium, and sodium salts respectively.

Table 1 Smoke Vapour Phase Constituents of Cigarette Smoke

Component	µg (fifth puff) Control	 8.3% $NaNO_3$
NO, NO_2	15.2	48.7
N_2O	1.0	11.0
HCN	13.2	23.3
CH_3CN	12.8	29.0

Data derived from Terrell and Schmeltz[4].

The cigarettes were smoked to 30 mm butts under standard conditions; sidestream and mainstream smoke were collected separately. The components were separated by standard gas chromatographic techniques and analysed by low voltage mass spectrometry.

Table 2 Mainstream Nitrogen Oxides from ^{15}N-Nitrate

Nitrate	Enrichment (%) Unsmoked tobacco	 N_2O	 NO
Calcium	86	89	ND
Sodium	89.5	87	ND
Potassium	ND	ND	85

Table 2 gives the ^{15}N enrichments of the nitrogen oxides in mainstream smoke. The similar enrichments observed in the nitrogen oxides and the precursor nitrates indicate negligible dilution from other sources.

Table 3 Sidestream Ammonia from ^{15}N-Nitrate

Cigarette	Sidestream NH_3 (mg)	Calculated % of NH_3 from NO^-_3	^{15}N atom %
Kentucky ref. 1R1	5.3	—	—
+K $^{15}NO_3$	8.3	36	35
+Ca $^{15}(NO_3)_2$	9.0	40	33
+Na $^{15}NO_3$	10.2	48	42

Table 3 shows: (*a*) the increased ammonia deliveries from the nitrated cigarettes over the delivery of the reference cigarette, (*b*) the percentage increase in ammonia which resulted from the added nitrate, and (*c*) the corresponding incorporation of ^{15}N into the ammonia. These data demonstrate for the first time the conversion of nitrate nitrogen to ammonia during the smoking process. The conversion is highly efficient: for example 25% of the nitrogen added as calcium nitrate was converted to measurable sidestream ammonia; the absolute conversion, of course, is somewhat higher. The conversion to ammonia indicates that much of the smoke chemistry of nitrate may be the chemistry of intermediary ammonia.

Ammonia was supplied to a Kentucky Reference cigarette in the form of ^{15}N-glycine (2% N added by weight). The delivery of 13.8 mg (as against 5.3 mg for a control cigarette) of ammonia by this cigarette confirms glycine as an ammonia source. Table 4 shows that amides, heterocyclics and nitriles were highly enriched in ^{15}N. Thus, nitriles which were shown by earlier workers to increase if nitrate is added to filler can be derived from ammonia. Amides and N-heterocyclics are shown tp be efficiently formed from ammonia and thus might be derived from nitrate as well.

Table 4 Smoke Products Derived from ^{15}N-Glycine

Smoke product	Delivery (µg cigarette)	^{15}N atom %
Ammonia	13,800	48
Acetamide	22	73
Pyrrole	263	54
Acrylonitrile	52	28
Hydrogen cyanide*	82	42

* Hydrogen cyanide data represent mainstream smoke. Other data are taken from the sidestream.

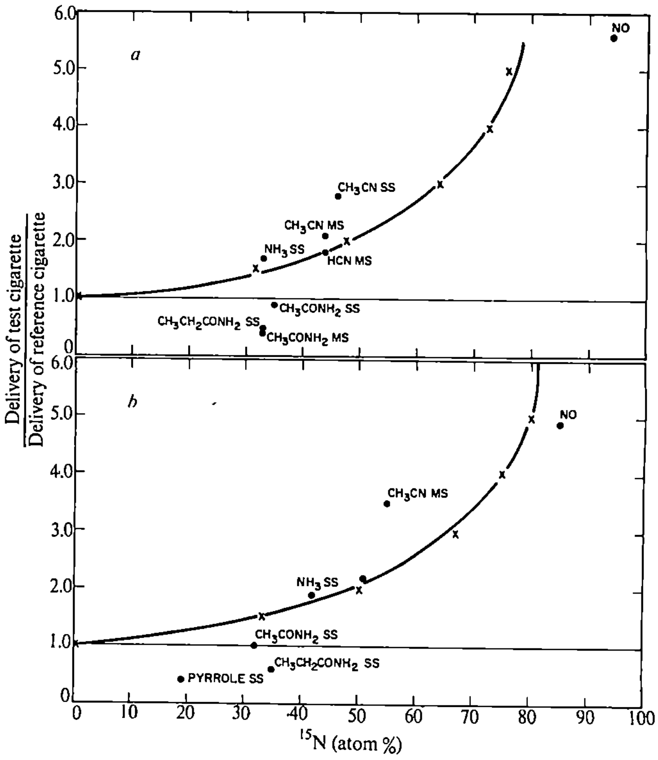

Fig. 1 Ratios of deliveries are plotted against ^{15}N uptake. Theoretical curve (—X—) would result if a 1 : 1 correspondence existed between ratios and uptake. SS and MS refer to sidestream and mainstream smoke respectively. *a*, Cigarette prepared with $Ca(^{15}NO_3)_2$; *b*, cigarette prepared with $Na^{15}NO_3$.

Table 5 shows that the amides were highly enriched when cigarettes containing ^{15}N-nitrate were smoked. The enrichments were found to be equal to or less than the corresponding ammonia enrichments, suggesting that the amides result from: (1) reduction of $^{15}NO_3$ to $^{15}NH_3$; (2) reaction of the ammonia with a suitable intermediate to produce the amides. The N-heterocyclics were also significantly enriched but to a lesser degree than were the amides. This is compatible with the *a priori* assumption that there are non-ammonia routes to these substances.

Table 5 ^{15}N-Enrichments of Compounds Formed during the Smoking of Cigarettes Treated with ^{15}N-Nitrate

Compound	Ca	^{15}N atom % K	Na
Formamide	34	—	—
Acetamide	34	25, 30*	33
Propionamide	34	24, 21*	38
Pyrrole	16	15	20
Methyl pyrrole	23	41	—
Dimethylpyrazine	17, 15*	17	24
Methyl pyridine	—	10	11
Pyridine	11, 13*	9	13
Hydrogen cyanide	39*	45*	58*
Acetonitrile	46, 45*	49, 42*	56, 56*
Acrylonitrile	35, 39*	39, 38*	56, 50*
Ammonia	33	35	42

* Asterisk numbers refer to mainstream smoke. All others refer to sidestream smoke.

Finally, the nitriles are shown to be dramatically affected by the nitrates; their enrichments being greater than those of ammonia, amides and N-heterocyclics, but less than those of the nitrogen oxides. Either or each of two processes super-posed on reactions going on elsewhere in the cigarette may account for these observations. First, NH_3 may be generated at a favourable temperature such that nitriles (or suitable nitrile precursors) are formed before NH_3 mixing can occur. Second, nitric oxide which is highly enriched may react with alkyl radicals to give oximes which can dehydrate to give nitriles.

The ratios of yields of nitrogenous products from calcium and sodium nitrate cigarettes to yields obtained from the reference cigarette are plotted against ^{15}N uptake in Fig. 1. Data points would be on the theoretical curve if a 1 : 1 correspondence existed between product formation and ^{15}N uptake. These figures show the extent to which other factors cause differences in yields between the experimental and control cigarettes. The position of hydrogen cyanide in the graphs indicates subtle differences between the behaviour of nitrates. Sodium nitrate, for example, affected the puff count more than did the calcium nitrate, presumably because of a greater oxidizing action. Deviations from the theoretical line might also be due to depletions of the "co-precursors" of the nitrogenous products.

The ability of nitrates to contribute nitrogen oxides and oxygen to smoke is well known. Their ability to influence smoke product distribution *via* a highly efficient production of intermediary ammonia has now been demonstrated.

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BIOLOGICAL SCIENCES

Foetal Antigens and Cancer

EVIDENCE from several independent sources[1-8] suggests that the neo-antigens of tumours that excite transplantation immunity are reawakened or derepressed foetal antigens, that is, are differentiation antigens that make a transient appearance early in foetal life.

If foetal tissues do indeed contain antigens capable of exciting cell-mediated immunity (CMI) then the growth of foetal tissues in normal adult syngeneic mice should be inferior to their growth in immunosuppressed mice. Moreover the experiments of Coggin and his colleagues[4-8] led us to expect that inoculation of foetal tissues into normal adult syngeneic mice would impede the growth of autochthonous or transplanted syngeneic tumours. For these reasons we decided to study the growth of foetal tissues in adult mice in various circumstances to learn whether an initial exposure to foetal tissue modified the behaviour of a second foetal implant or altered the growth of chemically induced or transplanted tumours.

Minced or mechanically homogenized tissues from 12-d-old CBA or C57 foetuses were implanted under the kidney capsule of adult mice. Recipients were killed at intervals and the sizes of the implants and the histological variety of tissues assessed.

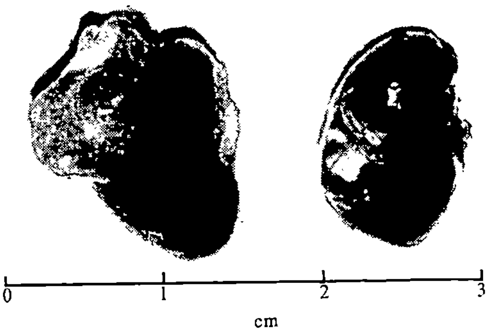

Fig. 1 Growth of homogenized 12-d CBA foetal tissues under the kidney capsule of normal adult CBA mice (right) or immunologically deprived CBA mice (left).

Recipients were either normal adult (8–12 weeks old), CBA males or thymectomized, lethally irradiated and bone marrow reconstituted ("deprived"[9]) mice of the same age, sex and strain. In some experiments recipients were "presensitized" to foetal tissues by the subcutaneous inoculation of foetal homogenates 2 weeks before the experimental implant.

Implants of both syngeneic and allogeneic foetal tissues grew poorly in normal adult mice. Implants of both types were about equally small and showed a restricted variety of tissues. In addition, a moderate lymphocytic infiltrate was observed in grafts of both kinds. The adult syngeneic tissue homogenates grew equally well in both environments, so it is unlikely that the two environments differed merely in their capacity to support growth as such. In deprived mice syngeneic and allogeneic tissues grew in great profusion to form embryomas (Fig. 1) in which most foetal organs or tissue types could be recognized (Table 1). Lymphocytic infiltration was absent.

Table 1 Histological Variety of Foetal Tissues growing under the Kidney Capsules of Normal Adult CBA Mice, Immunologically Deprived CBA Mice, and Normal Adult CBA Mice Pre-Treated with CBA Foetal Tissues

Normal mice Six mice	Deprived mice Six mice	Pretreated mice Six mice
Cartilage	Cartilage	Cartilage
Skeletal muscle *	Ossifying bone	Mature bone
Squamous epithelium *	Mature bone	Squamous epithelium
	Squamous epithelium	Hair follicles
	Hair follicles	Tooth
	Sebaceous gland	Kidney
	Eye	Ciliated epithelium
	Liver	Adipose tissue
	Ciliated epithelium	Pancreas
	Intestine	Skeletal muscle
	Brain	
	Marrow	

For details, see text.
* In one mouse only.

Superior growth of syngeneic foetal tissues in deprived mice was recorded in six out of eight sets of experiments but not in the other two; evidently some variable influencing the results has not yet been identified.

In normal mice which had received a previous implant of syngeneic foetal tissue, second foetal implants grew larger and in greater histological variety (Table 1).

An unexpected finding was that, far from suppressing tumour induction by 20-methylcholanthrene the previous treatment of adult mice with living syngeneic foetal tissues advanced the time of appearance of tumours (Fig. 2). Pretreatment with foetal implants also enhanced the growth of a transplantable syngeneic tumour (Meth A) in normal adult Balb/c mice. This effect was more marked when irradiated

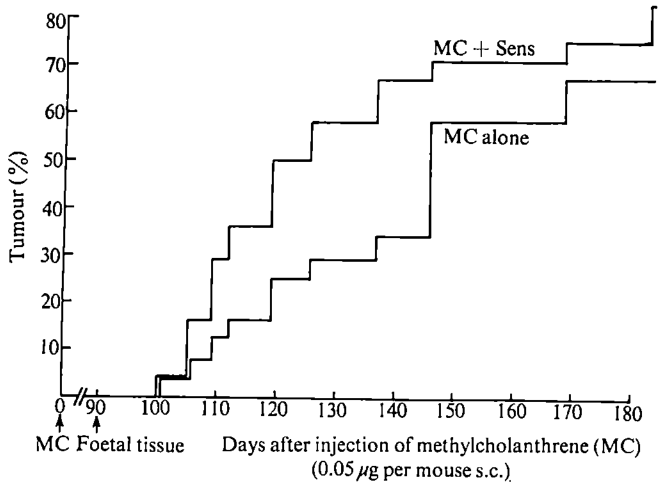

Fig. 2 Inception times of methylcholanthrene-induced tumours in normal adult CBA males, and in males pretreated with CBA foetal tissues.

allogeneic (C57) inocula were used (Fig. 3) and less striking with the use of non-irradiated syngeneic foetal tissue (Fig. 3).

These results suggest that syngeneic foetal antigens (differentiation antigens) may provoke a transplantation reaction in competent adult mice. Two observations accord with an immunological interpretation of these findings: the superior growth in immunologically deprived mice and after "presensitization"; correspondingly, the growth of transplanted tumours and the tumour induction rate after exposure to a chemical carcinogen were improved or "enhanced" in mice previously inoculated with foetal tissues. Although this was a result in the wrong direction from the standpoint of any possible therapeutic application, the modification of a second response by a first points to some immunological affinity between foetal and malignant tissues. In our models conditions have yet to be found in which tumour growth is inhibited. Irradiation of the foetal inocula was found to be essential by Coggin and his colleagues[4,6,7] and Prehn[10,11] has shown that in some circumstances a mild degree of immunity may enhance not only tumour but also foetal growth. These and other possibilities are now being investigated.

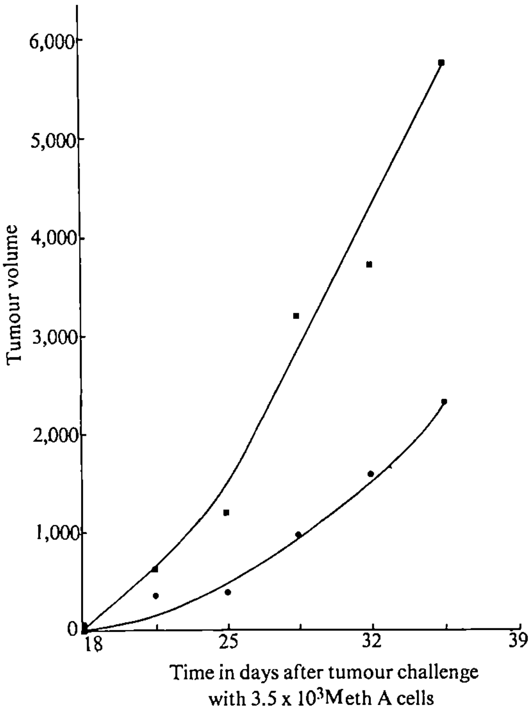

Fig. 3 Growth rate of 3.5×10^3 Meth A tumour cells in normal adult Balb/c mice (●—●) and in Balb/c mice pretreated with allogeneic foetal tissues (■—■).

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Inhibition by Insulin of Cardiac Cathepsin D Activity

In previous studies of foetal mouse hearts in organ culture, I have demonstrated that insulin improves net protein and amino acid balance[1] and prolongs the duration of survival and beating *in vitro*[2]. The hormone exerts its action on amino-acids such as phenylalanine whose metabolic pathways in the heart are confined almost exclusively to protein synthesis and degradation, as well as on those such as valine and alanine that may enter a variety of alternative pathways as well.

The effects of insulin on net protein balance could be a consequence simply of the well known ability of the hormone to improve protein synthetic capacity[3]. An alternative explanation is that insulin might inhibit the rate of protein degradation. Such an effect has been demonstrated in the liver[4], and preliminary experiments by Rannels *et al.*[5] have suggested that a similar action may occur in isolated hearts. No biochemical mechanism has been demonstrated, however, by which insulin can inhibit proteolysis. Perhaps there is a simple decrease in proteolytic enzyme activity, but there has been no evidence that the hormone has such an influence on any of the major proteases. Accordingly, I carried out the experiments described here to determine whether or not insulin has any effect on cathepsin D, a lysosomal enzyme that provides almost all the acid protease activity in the mouse heart[6].

Intact spontaneously-beating hearts from 17 to 21-d foetal mice were maintained in organ culture. The hearts, each of which weighed only 1–3 mg, were pooled in matched groups of ten to provide sufficient material for the assays.

As before[1], cardiac weights and total protein content were significantly greater in hearts exposed to insulin; changes in the two parameters were proportional, so that the concentration of protein was similar in the control and treated groups (96 ± 2.4 (SEM) mg g^{-1} in controls; 97 ± 3.6 mg g^{-1} in those exposed to insulin). Activity of creatine phosphokinase, a myocardial enzyme that is not concerned with proteolysis and

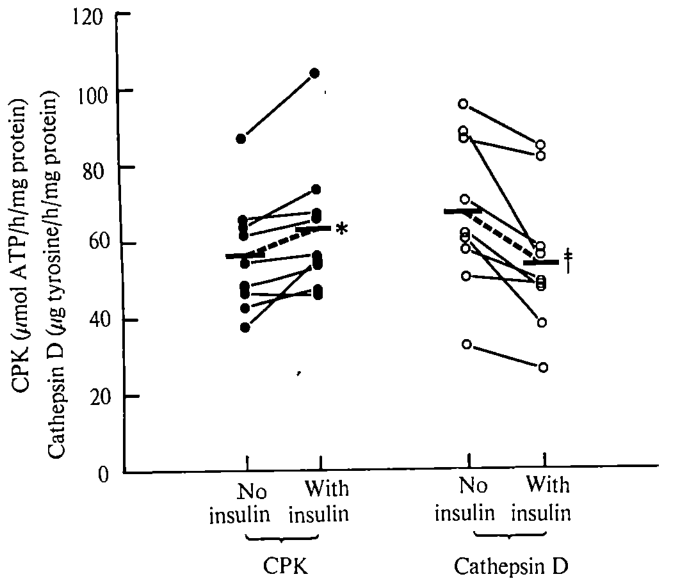

Fig. 1 Activities of creatine phosphokinase (CPK) and of cathepsin D in the presence or absence of insulin. One group of ninety hearts was maintained for 2 to 3 d in "medium 199" (ref. 7) with no insulin; matched litter mates were maintained in an identical medium supplemented with 50 μg ml^{-1} of purified bovine insulin. After 2 to 3 d the hearts were weighed, homogenized, and analysed for cathepsin D (ref. 8), creatine phosphokinase[9], and protein[10]. Lines connect the values obtained in simultaneous assays of matched hearts. Horizontal bars denote mean values for each group. * $P<0.05$ compared to matched group (Student's "*t*" test for paired observations); ‡ $P<0.01$.

that is not lysosomal in origin, was slightly greater in the insulin-treated group (Fig. 1).

In contrast, activity of cathepsin D was significantly higher in hearts that had been deprived of insulin (Fig. 1). In the absence of the hormone cathepsin D activity was 27 ± 6.8% greater than in its presence ($P<0.01$). Separation of the homogenate in 0.25 M sucrose into a fraction that was bound to sedimentable particles and a fraction that was free in the supernatant[11] revealed that a greater change in enzyme activity occurred in the "free" fraction than in the "bound" compartment. The ratio of free to total activity, which is believed to reflect the fragility of lysosomal membranes, rose from 61 ± 0.5% to 64 ± 0.5% in the absence of the hormone.

These experiments demonstrate for the first time that insulin can affect cathepsin D activity in the heart. The effect of the hormone is to reduce the total synthesis or specific activity of the enzyme, and not solely to alter the physical characteristics of the lysosomes. Although the exact role of cathepsin D in mediating intracellular proteolysis remains to be established[12], the results suggest the possibility that the ability of insulin to improve net protein balance in hearts maintained for long periods *in vitro*, as well as its ability to prolong the duration of survival and function of isolated myocardium, may be related in part to a reduction in lysosomal proteolytic activity.

The present findings may be a curiosity of the specific conditions of the experimental system, and they may have little or no applicability to physiological or clinical problems. But the association of insulin deficiency with increased activity of lysosomal proteins and with increased proteolysis might well be a general phenomenon of wide importance. In this regard it is of special interest that starvation, which among its other effects causes hypoinsulinaemia, has recently been shown to be associated with markedly increased activity of cathepsin D in myocardial cells[6]. That observation, plus the present results, suggests that analyses of cathepsin D activity in other conditions associated with alterations in insulin levels *in vivo* would be important. Also, because preservation of isolated hearts and other organs constitutes an important problem in the field of clinical organ transplantation, the role of insulin in inhibiting the activity of proteolytic enzymes deserves evaluation as a possible mechanism for delaying deterioration of organs maintained *in vitro* before transplantation.

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Milk Ejection during Alcohol Anaesthesia in the Rat

THE inhibition of oxytocin release by alcohol during labour and milk ejection has received wide acceptance and studies purporting to show this inhibitory function have been conducted on the rabbit[1], woman[2] and the rat[3]. In the last and most recent study, the litter weight gain was measured after a timed exposure to the alcohol-treated doe. Such criteria have, however, been outdated by recent developments. Milk ejection in the rat, and thus oxytocin release, is not the simple reflex process observed in other mammals[4–9]. In particular, milk ejection does not occur with the onset of nursing and, even when it does commence, it is an intermittent and an all-or-none event. Furthermore, no anaesthetic has so far been found which blocks this neuroendocrine reflex in the rat. In the light of these developments, therefore, I re-examined the alleged "ethanol block to oxytocin release" and found that the rat, completely anaesthetized with ethyl alcohol, displayed an almost normal pattern of milk ejection.

Adult rats were taken at day 10 of lactation and separated from their litters for 18 h (refs. 3–8). The does were then anaesthetized with 5 g kg^{-1} ethyl alcohol (12% solution) given by stomach tube or i.p. injection. When the righting reflex was abolished, usually within 15 min of the alcohol treatment, each doe was placed supine on the observation table and ten pups applied to the nipples. Milk ejection commenced, as judged from the behavioural activity of the young[4–7], 50.9 ± 5.6 min after the alcohol administration or about 30 min

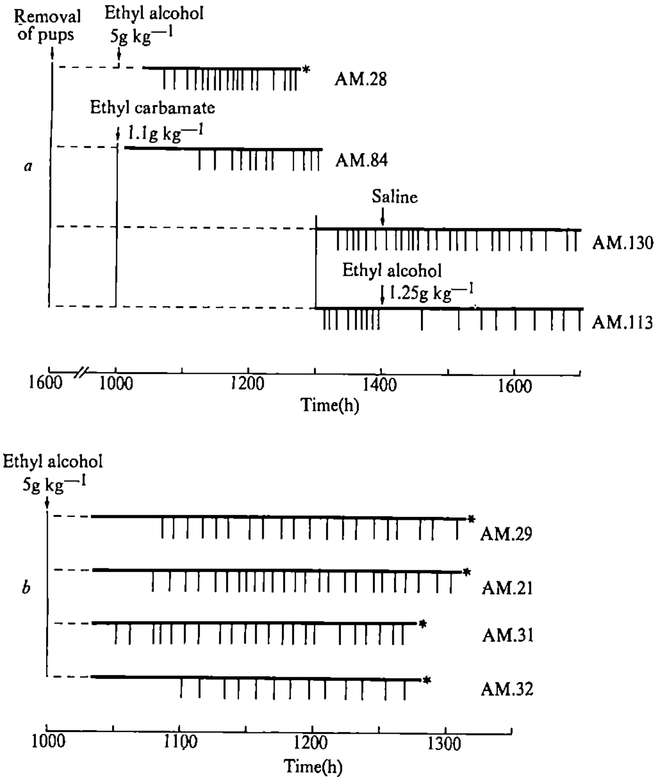

Fig. 1 *a*, Typical pattern of milk ejection is shown for each treatment; the code for the animal concerned is given on the right. The "thick" horizontal bar corresponds to the period of suckling and each vertical deflexion beneath this bar represents an individual milk ejection. *b*, Four other examples of milk ejection during anaesthesia with ethyl alcohol. In all cases of alcohol anaesthesia the recording was terminated by the doe attaining a righting reflex (✻).

after the pups were applied to the nipples. Thereafter, a regular and intermittent pattern of milk ejection was observed for 2–3 h (Figs. 1 and 2). The mean milk ejection interval, calculated from the first milk ejection to the time at which the righting reflex was re-established, was 8.14±0.73 min. The modal interval was shorter at 5–6 min as the distribution was made asymmetrical by the occurrence of a few rather long intervals of 20 min or more. On average 19.8 milk ejections were displayed by each preparation for a milk yield of 9.0±1.2 g. This pattern of milk ejection is almost identical to that observed during anaesthesia with pentobarbitone sodium (20–40 mg kg^{-1}), ethyl carbamate (1.1 g kg^{-1}) and tribromoethyl alcohol (175–325 mg kg^{-1}) (ref. 6). Milk ejection in the conscious doe differs in only two respects; the latency to the first milk ejection is shorter at 10.8±3.1 min and the duration of nursing is limited by the doe to periods of 20–40 min. The mean milk ejection interval in the conscious doe, excluding the long periods when the doe was away from the nest, was 6.6±0.6 min.

The behavioural response of the pups consists of a synchronized extensor or stretch reaction in which all the young suck and pull vigorously at the nipple for about 10 s. This is not the cue for oxytocin release, for it always follows, albeit within a second, the rise in intramammary pressure[6–7]. Recordings of intramammary pressure were obtained during suckling of the doe under anaesthesia with ethyl alcohol at 5 g kg^{-1} and this correlation between behaviour and intramammary pressure was confirmed (Fig. 2). Each milk ejection was the result of an extremely uniform release of oxytocin. In the example in Fig. 2 the release was 0.4–0.6 mU oxytocin, but in other animals a release of up to 1.5 mU oxytocin per milk ejection was observed. Fig. 2 also illustrates two other features of the milk ejection reflex of the rat which have not previously been described. First, the administration of exogenous oxytocin in physiological doses of 0.1–1.0 mU caused no observable change in the regular pattern of endogenous hormone release, by contrast with the many claims that exogenous oxytocin inhibits the reflex release of this hormone from the neurohypophysis[10–15]. Second, there was a threshold level of intramammary pressure below which the behavioural response of the pups was not observed. Thus injections of less than 0.2 mU oxytocin failed to elicit a behavioural response though there was a distinct rise in intramammary pressure. The young presumably apply continuous negative pressure to the nipple and can taste milk after it arrives in the main teat duct at the time of milk ejection. It is only to be expected therefore that a small and rather transitory milk ejection will fail to elicit a behavioural reaction from the pups. The endogenous release of oxytocin is only two to four times the threshold for a behavioural response and it is feasible that on occasions the reflex release of oxytocin might fall below the threshold for an effective milk ejection. Such subthreshold releases of oxytocin, less than 0.2 mU, were not, however, observed during any of the pressure recordings.

Studies with less than the full anaesthetic dose of ethyl alcohol are complicated by several factors. With a conscious, though tranquillized, doe there is the problem of inhibition emanating from higher neural centres—the so called "cortical inhibition"[16]. It is also difficult to control the most critical factor in the milk ejection reflex, the suckling stimulus. These problems were largely overcome by the administration of ethyl alcohol to does already anaesthetized with ethyl carbamate (urethane). Anaesthesia with 1.1 g kg^{-1} ethyl carbamate was most prolonged and 24 h after treatment the does were still

Fig. 2 Continuous polygraphic record of the intramammary pressure of a rat suckled when anaesthetized with ethyl alcohol at 5 g kg^{-1}. Reflex milk ejection (RME) is shown in series with milk ejection elicited by the intravenous administration of oxytocin, the dose of which is given in mU immediately prior to the milk ejection concerned. The time (min) is recorded from the application of the anaesthetic. Ten pups were applied to the nipples at approximately 90 min and the recording was subsequently terminated at 200 min by the doe attaining a righting reflex. The stretch response of the pups is signified by ● immediately beneath the pressure trace. The intramammary pressure associated with RME is equivalent to about 10–15 mm Hg.

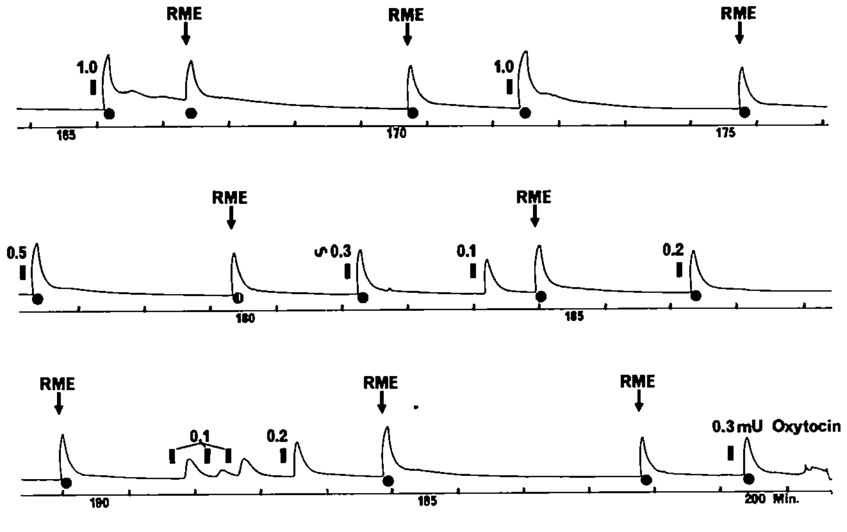

Table 1 Effect of Ethyl Alcohol on the Incidence of Milk Ejection*

No. of rats in sample	No. of milk ejections/rat in the 30 min before treatment	Treatment with ethyl alcohol g kg^{-1}, i.p.	No. of milk ejections/rat/30 min post-treatment						Total No. of milk ejections/rat	Weight gain/ litter g
			0–30	30–60	60–90	90–120 min	120–150	150–180		
4	4.8	2.50	0	0	0	0	0	0.3	8.0	4.3
5	5.0	1.25	0	1.0	1.4	1.8	2.4	2.8	16.8	6.7
6	4.7	0.625	1.8	2.2	2.5	3.7	3.8	2.8	24.0	9.4
4	5.0	Saline	4.5	4.0	3.5	2.8	2.0	2.7	27.0	8.3
14	5.0	—	4.8	3.8	3.9	2.4				

* Applied to the nursing rat under anaesthesia with ethyl carbamate, 1.1 g kg^{-1}. 3 h later the pups were applied to the nipples and then, 1 h later, the alcohol was administered.

ataxic. The latency to the first milk ejection was 80.2 ± 6.7 min when the pups were applied within a few minutes of induction of anaesthesia, but this dropped to less than 20 min when the pups were applied 3 h later (Fig. 1). Doses of 0.625–2.50 g ethyl alcohol kg^{-1} were then administered by i.p. injection to this latter preparation 1 h after the application of the young, that is after the occurrence of four to ten milk ejections. The effect of the 2.5 mg treatment was to inhibit milk ejection in the subsequent 3 h of suckling (Table 1); with the 1.25 and 0.625 mg treatments the block was less complete and the first milk ejections occurred after 66 and 8 min, respectively. The frequency of milk ejection then increased with time and after 2–3 h the frequency of milk ejection was greater than that observed in the control preparations.

My study does not completely contradict the former work on the interaction between alcohol and oxytocin release. If I had applied the criteria of Fuchs[3], that is, the weight gain of the litter after 30 or 60 min exposure to the alcohol-treated doe, an increase of weight would not have been observed in most preparations. An almost normal pattern of milk ejection, however, occurred throughout the remaining 2–3 h of anaesthesia. The increased latency to the first milk ejection, compared with conscious preparations, is not specific to alcohol, for it is seen with all the anaesthetic situations so far studied[6]. It is possibly related to the metabolism of the drug concerned for, first, it is dose dependent, and, second, it is reduced to the value observed in the conscious doe when the young are applied sometime after the onset of anaesthesia. Conversely, milk ejection is not confined to a short period coincident with anaesthetic recovery. Considerable evidence is available to suggest that full anaesthesia is a prerequisite for milk ejection under the experimental circumstances which have been studied. Milk ejection was never observed during periods in which the doe displayed positive signs of consciousness, for example, head, limb and whisker movements. The cortical electroencephalogram (EEG) was recorded from both alcohol and urethane treated does: milk ejection occurred during slow-wave conditions (synchronized-EEG[17]) but never during arousal (desynchronized EEG). One possible explanation is that full anaesthesia, as expressed in the form of a sleep-like EEG, eliminates the cortical inhibition of the milk-ejection reflex which would otherwise operate. The possibility of there being a stress effect, involving the pituitary-adrenal or sympathetic-adrenal systems, is slight, for most animals are severely stressed throughout the period of anaesthesia, and this is particularly true after the administration of urethane[18].

I conclude therefore that the "ethanol block to oxytocin release" is seen as a relatively ineffective and non-specific phenomenon.

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Lamprey Growth and Biological Conditions in the Bristol Channel Region

IN view of public disquiet over the possible deterioration of some of our rivers and coastal waters, including the Severn estuary, it is encouraging to find some evidence, although indirect, that as far as the populations of migratory lampreys are concerned, conditions in the area of the Bristol Channel appear to be at least as favourable for their survival and growth as was the case ten years ago.

In those major river systems in which they occur, the anadromous lampreys are generally much more numerous than the migratory salmonids and, for this reason alone, are a more useful biological indicator of changing conditions in the estuarine and coastal environment. As predaceous animals feeding on a wide variety of teleost fishes it would be anticipated that the rate of growth of the adult lamprey would, to some extent, reflect the abundance and accessibility of these host species within their feeding area. This is borne out by experience in the Great Lakes of North America where the body size of spawning migrants of the landlocked sea lamprey, *Petromyzon marinus*, has proved to be a sensitive index of the status of the host fish populations[1]. Following the introduction of these parasitic lampreys into the Upper Great Lakes, their body size has tended to decrease over the period of time for which adequate records are available and this has been most marked in Lake Huron, where the average weight and length decreased by 35% and 13% respectively between 1947 and 1960. That this decrease in body size was at least partly attributable to a depletion of host fishes is suggested by the marked decline in the stock of lake trout (*Salvelinus*

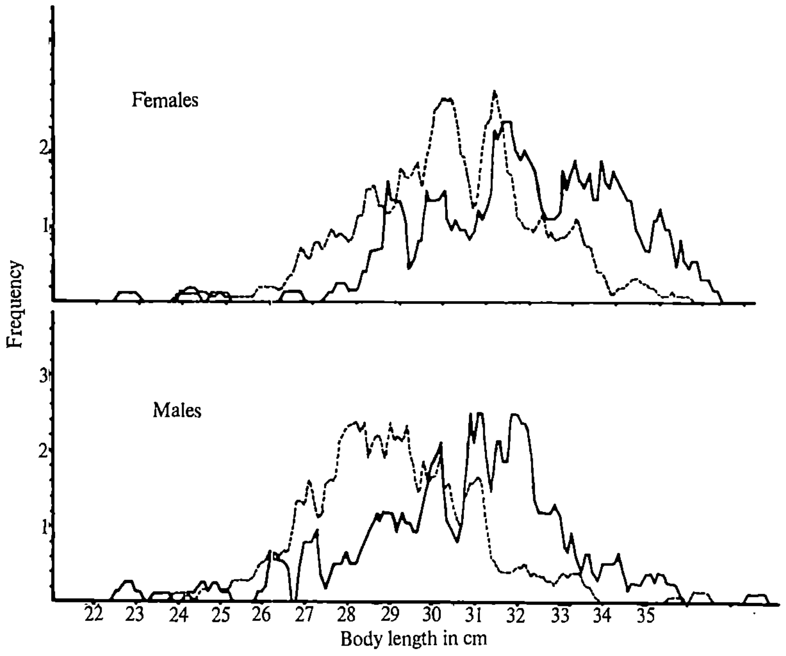

Fig. 1 Length-frequency distributions for male and female river lampreys (*Lampetra fluviatilis*) taken from the River Severn in October 1962 and 1971. (Smoothed by moving average of 5 mm.) - - -, October 1962; ——, October 1971.

namaycush), burbot (*Lota lota*) and whitefish (*Coregonus clupeaformis*) that was already apparent before the maximum number of lampreys was reached in 1949.

Insofar as they reflect a dynamic interrelationship between changing parasite and host populations within a closed lake system, these changes may be regarded as exceptional. Nevertheless, they indicate that the final body size attained by the sexually mature lamprey is mainly a reflexion of feeding opportunities and that a deterioration in feeding conditions and growth rates is apparently not accompanied by an extension of the parasitic phase of the life cycle.

The upstream migration of river lampreys (*Lampetra fluviatilis*) in the Severn generally begins in late summer or early autumn, reaching a peak in October or November. In the autumn of 1962 and 1971, records have been made of the lengths and weights of more than 1,300 river lampreys (Table 1), most of which were caught at Tewkesbury. The length (Fig. 1) and weight frequency distributions show the wide dispersion and polymodality characteristic of this species[2] with lengths varying from 22.7 to 37.6 cm and weights from 19.3 to 105.0 g. For both sexes, the body size of the 1971 migrants was conspicuously larger than in 1962. Thus, the mean length for males caught in October 1971 was 30.6 cm compared with only 29.0 cm in 1962, whereas for females the corresponding values were 31.8 cm and 30.1 cm, representing percentage increases of 5.5% and 5.6% respectively. Differences in body weight are even more striking; for 1971 the mean weight for October females is 69.1 g compared with 58.0 g in 1962: for the males the corresponding figures are 59.1 g and 51.9 g, giving percentage increases of 19.1 for females and 13.9 for the males. Similar differences were also observed in the November material. For each of the corresponding groups in Table 1 the differences in the means are highly significant, with probabilities in no case greater than 0.001.

It is difficult to assess how far changes in body size might be a result of fluctuations in the relative abundance of the lamprey population and consequent changes in the pressure on host fish populations. Nevertheless, such evidence as we have suggests that this could not account for the difference in body size of the 1962 and 1971 populations; indeed, according to reliable local observers, the 1971 run was one of the largest within recent years; a view substantiated by the number of lampreys caught.

The precise limits of the feeding area of the river lamprey are unfortunately not known. After studying their osmoregulatory ability in seawater, Bahr[3] has concluded that in the North Sea coastal areas this species is predominantly an estuarine feeder, although more recent work on the downstream migrant stages has indicated that in many cases they can be acclimated to full strength seawater[4,5]. In the Mediterranean, Zanandrea[6] obtained river lampreys within a few km of the coast in the area of the Gulf of Gaeta and all the indications from the North Sea and the Baltic suggest that this species probably feeds in the coastal regions[2]. Thus, it seems likely that the populations which migrate into the Severn estuary are probably drawn from the area of the Bristol Channel and its immediate offshore shallow waters, and that in these regions the supply of suitable host fishes is at least as adequate as in 1962. In this connexion a recent report[7] has indicated a significant revival in the stocks of certain teleosts in the

Table 1 Mean Lengths and Weights of River Lampreys in 1962 and 1971

	Males			Females		
	Number	Length, cm. Mean ± s.e.	Weight, g Mean ± s.e.	Number	Length, cm. Mean ± s.e.	Weight, g. Mean ± s.e.
October 1962	340	29.0 ±0.1	51.9 ±0.6	348	30.1 ±0.1	58.0 ±0.6
November 1962	43	29.0 ±0.3	48.5 ±1.4	70	29.8 ±0.2	54.6 ±1.3
October 1971	153	30.6 ±0.2	59.1 ±1.2	154	31.8 ±0.2	69.1 ±1.2
November 1971	150	31.5 ±0.2	55.0 ±1.0	150	32.5 ±0.2	62.4 ±1.1

English Channel, in particular, the cod (*Gadus morhua*), ling (*Molva molva*) and to a lesser extent the herring. Should similar changes have occurred within the areas adjacent to the Bristol Channel they may well have produced conditions more favourable to the growth of the river lamprey. This seems to conflict with conclusions reached in a recent survey of coastal sport fishing in Britain[8], which suggested that the Bristol Channel area and its approaches had suffered a greater decline in the variety of fish than most other coastal waters. The explanation may be that the adverse effects of pollution are more apparent in the immediate coastal zone (where angling is generally carried out), rather than further offshore where the lamprey is believed to feed.

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Comment on "Visual System's View of Acoustic Space"

MORRELL[1] has presented "evidence of a rigorous spatial specificity in the acoustical projection to visual neurones". He observed that "along the horizontal meridian, the acoustical receptive fields were arranged in as orderly and systematic a way as the receptive fields for vision". The acoustical receptive fields were mapped onto visual receptive fields "having the same horizontal boundaries as the corresponding visual receptive field(s)" (Fig. 4, ref. 1). The superposition of the visual and auditory receptive fields is difficult to understand if one takes into account that the eyes move within their orbits. What happens to this superposition if, for instance, the eyes are turned 10° to the left side whereas the head remains in its original position? Three different results are conceivable: (1) The visual receptive field is shifted 10° to the left and thus keeps its position with reference to retinal coordinates. The auditory receptive field remains at its original position. In this situation there would be a dissociation between the position of the visual and the auditory receptive fields. (2) Both the visual and the auditory receptive fields are shifted 10° to the left, that is, the visual system would "control" the position of the receptive fields of the auditory system. (3) Both the visual and the auditory receptive fields remain at their original position, that is, the position of the visual receptive fields is remapped in order to coincide with the position of the auditory receptive fields, and thus with head coordinates. The outcome of an experiment investigating these possibilities cannot be anticipated, and the results might differ between an anaesthetized and an unanaesthetized preparation. It seems, therefore, that the data do not yet establish clearly that there is a "rigorous spatial specificity in the acoustical projection to visual neurones".

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Reply

COMMENTING on my communication "Visual System's View of Acoustic Space"[1], Pöppel has made two observations. The first argues that I have not demonstrated a "rigorous spatial specificity in the acoustical projection to visual neurones" as that statement does not take into account what may happen when the visual axis is displaced from the auditory. The second comment suggests three different findings which such displacement might yield. Pöppel admits that there is little basis for prediction among these possibilities and that the result might differ depending on whether or not the animal was anaesthetized.

The questions are good. The answers depend on further experimentation. My conclusion was based on findings in a particular and well defined experimental situation; one in which the acoustical and visual axes were aligned. Given those restrictions, I feel the conclusions to be warranted. I do not extend them to all and every circumstance. Given the experimental situation as defined, the data seem to have an extraordinary regularity—one which is in every way as regular as the receptive field properties for visual stimuli.

Experiments to answer the questions posed by Pöppel are not yet complete. Preliminary findings indicate, however, that shifting the acoustical axis, for instance, by closing one ear, while maintaining fixed retinal coordinates results in a shift of the acoustical receptive field but not the visual. Thus, the third possibility mentioned by Pöppel does not obtain; the acoustical system does not "control" or remap the visual receptive field. Nor does the reverse occur. Fixation of retinal coordinates does not impose similar fixation of acoustical fields. Dissociation, that is, the first of Pöppel's alternatives, seems to hold in the paralysed, unanaesthetized cat.

The implication of this finding with regard to the behaving, unrestrained animal remains to be determined.

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Attached *Sargassum* Found in Britain

ON February 17, 1973, some thirty scattered plants of the brown algal genus *Sargassum* were found attached on a sheltered shore in Hampshire. This is the only record for attached plants of a species of *Sargassum* in Britain. The specimens were well grown, attaining a length of up to 1 m (Fig. 1), and showed the characteristic morphology of this genus with monopodial branching, air-bladders and leafy laterals. We are confident that these plants constitute a recent introduction as the area has been under surveillance since 1967, being one of the localities for a possibly-introduced *Grateloupia* sp. (red alga)[1].

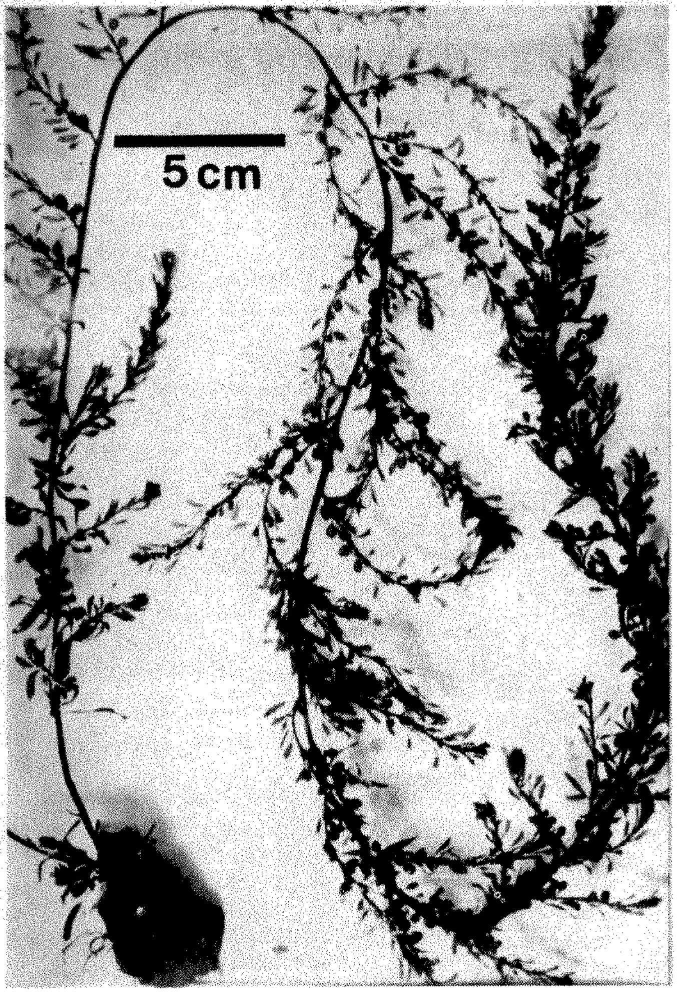

Fig. 1 Photograph of an entire attached plant of *Sargassum muticum.*

Sargassum is a taxonomically complex genus accredited with up to 250 species. From the available literature and herbarium specimens our plants were identified as *S. muticum* (Yendo) Fensholt. The occurrence of this species raises wider issues than merely the addition of another species to the British marine flora. Scagel[2] reported the accidental introduction of *S. muticum* from Japan into the northeastern Pacific with spat of the Japanese oyster (*Crassostrea gigas*). This alien weed is now distributed from British Columbia to California[3,4] and may be displacing the ecologically important local eel-grass (*Zostera marina*)[5]. Our *Sargassum* population is similarly associated with a *Zostera* bed. The outbreak of "wasting disease" of *Zostera* in the North Atlantic during the 1930s resulted in many important members of the *Zostera* community also being affected[6]. The implications of the introduction of a potentially serious competitor to the *Zostera* are self-evident.

Druehl[5] recently drew attention to the increasing practice of long-range transplantation of marine organisms, particularly those of commercial importance. He referred to the general lack of international control over this, despite inability to forecast reliably the effects of alien species on indigenous communities. Druehl also predicted the establishment of *S. muticum* in the eastern Atlantic as a result of direct importation by air of *C. gigas* from British Columbia into French waters. The accuracy of his prediction has been substantiated by the discovery reported here, made during the month following publication of his letter.

Spat of the Japanese oyster have been imported in bulk into French waters since 1966 from both Japan and British Columbia[7]. Importation of all molluscan shellfish into Britain is limited under the Molluscan Shellfish (Control of Deposit) Order of 1968, No. 1164. As far as is known, no *C. gigas* has been imported into British waters except under quarantine hatchery conditions; these were applied to the *C. gigas* spat reared in Chichester Harbour[8].

It is not possible therefore to conclude at present that the deliberate commercial spread of the Japanese oyster has been responsible for the accidental dispersal of *S. muticum* into the Channel, although this type of situation was envisaged by Elton[9], "But the greatest agency of all that spreads marine animals to new quarters of the world must be the business of oyster culture. . . ." Certainly a number of undesirable predators, for example, the American oyster-drill (*Urosalpinx cinerea*)[10], and competitors such as the slipper-limpet (*Crepidula fornicata*)[11], are known to have been introduced along with oysters.

Alternatively, other agencies, such as shipping, may have been responsible for the sudden appearance of *S. muticum.* Previous introductions into the Solent area which have become established include the Australasian barnacle *Elminius modestus*[12] and the Japanese sea-squirt *Styela clava*[13]. Other recently discovered seaweeds include three red algae—the *Grateloupia* mentioned above[1], another species of *Grateloupia*[14], and a *Callophyllis* species (unpublished). These are currently under investigation; all could have originated from the eastern Pacific seaboard.

We would like to take this opportunity of endorsing the opinion of Dr Druehl that "the scientific community must assist in formulating reasonable regulations which will apply to the introduction of exotic marine species for scientific or economic purposes".

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Ufasomes are Stable Particles surrounded by Unsaturated Fatty Acid Membranes

THE arrangement of lipid molecules in biological membranes enables them to play both a structural part in providing a matrix for membrane proteins and a functional one, in which they act as a barrier to the free flow of solutes[1]. These roles are usually fulfilled by phospholipids arranged in a flat bilayer with the hydrophobic segments of the molecules orientated towards the interior of the bilayer. The importance of this orientation is recognized in studies of model lipid membranes. Much of the knowledge of membrane lipid function has come from work with aqueous dispersions of liposomes, or microscopic phospholipid particles, in which the molecular arrangement is similar to the orientation found in biological structures[2].

We have been interested in testing the hypothesis that membrane lipids may constitute a primary target in the initiation of radiation injury in higher organisms[3]. Part of the test requires analysis of chemical changes in irradiated membranes which may be correlated with alterations in function. Studies with intact membranes are hampered by their great chemical complexity and even liposomes made from natural phospholipids are too heterogeneous chemically for the ready unravelling of reactions which follow irradiation.

To overcome these difficulties we tried to form structures similar to phospholipid liposomes but made from pure long-chain fatty acids. Thin layers of various acids and acid mixtures were deposited in glass vessels by evaporation of chloroform solutions and the film resuspended by hand or mechanical shaking in water buffered at *p*H 8–9. The suspensions contained spherical particles visible under a microscope but showing no fine structure. The spheres were unstable to drying and the technique of negative staining for electron microscopy which works well for liposomes[4] was unsuccessful. Freeze-etched specimens, however, showed clearly under an electron microscope that the spheres consist of an aqueous interior surrounded by one or more concentric layers of fatty acid membranes. A sphere made from oleic acid, shown in Fig. 1*a*, has at least twelve layers and resembles closely the electron micrograph of a phospholipid preparation published by Fluck *et al.*[5]. Some spheres have complex interior structures with many orientated sheets of incomplete membranes and others consist of collections of small spheres within a common envelope (Fig. 1*b*).

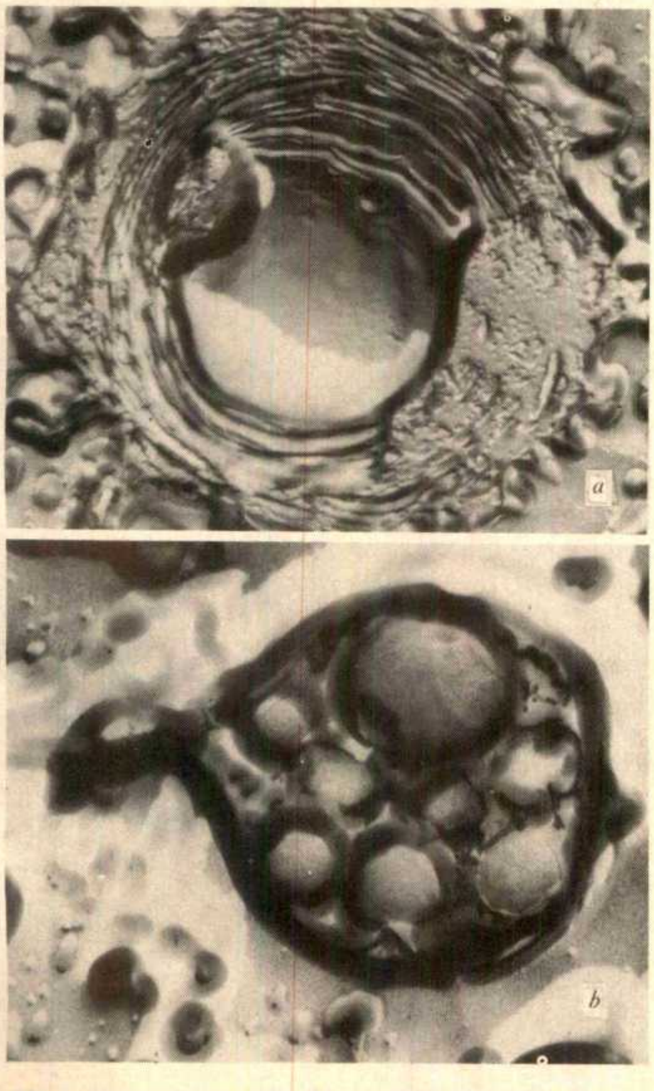

Fig. 1 Electron micrographs of freeze-etched oleic acid spheres (×41,650). *a*, A central aqueous region surrounded by concentric membranes—the flat pitted area was cut by the microtome. *b*, Smaller spheres enclosed by a common envelope.

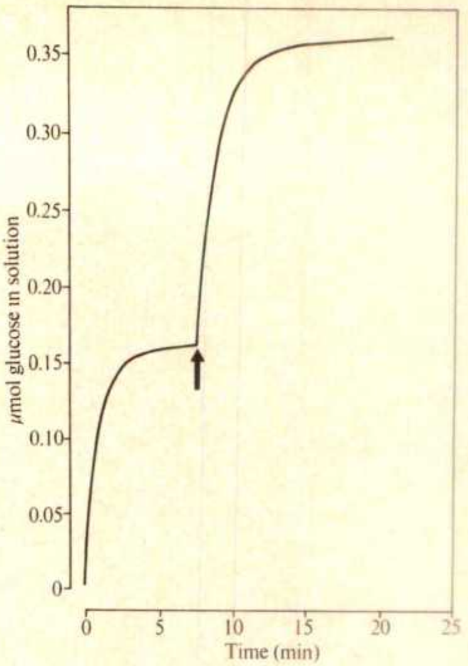

Fig. 2 Glucose retention by oleic acid spheres in 2.8 ml. of test solution (see text). The 0.17 μmol level corresponds to external glucose remaining after dialysis; the additional glucose was released when the spheres were disrupted with 'Triton' X-100, added at the arrow.

From the behaviour of fatty acids at interfaces[6] it can be inferred that the membranes consist of double layers of molecules orientated with their hydrocarbon tails towards the interior of the membrane and the carboxylic groups in the membrane surface. The separation between individual bilayers varies between 20 and 30 nm (Fig. 1*a*). This suggests that here, as in liposomes, aqueous shells are interposed between individual membranes. The bilayers are stabilized by ionization of a small proportion of the weak acid groups at the slightly alkaline *p*H. Because of the obligatory presence of unsaturated fatty acids we have called the particles ufasomes.

The presence of aqueous spaces within the ufasomes was confirmed by forming them in the presence of glucose, removing exterior glucose by dialysis, and measuring the amount of sugar released by spheres disrupted with 'Triton' X-100 detergent. The spectrophotometer trace shown in Fig. 2 was obtained with ufasomes made from 2 mg of oleic acid shaken with 0.1 ml. of *p*H 8 Tris buffer and 0.1 ml. of 1 M glucose. After dialysis for 4 h, 0.05 ml. of the suspension was diluted into 2.8 ml. of buffered aqueous solution containing NADP, ATP Mg^{2+}, hexokinase, and glucose-6-phosphate dehydrogenase in amounts recommended by Demel *et al.*[7].

The curve shows increase in optical density at 340 nm due to formation of NADPH in the presence of glucose. A correction was made for change in light scattering when the suspension cleared on addition of detergent. Clearly, the ufasomes held glucose in compartments inaccessible to the enzymes until their membranes were disrupted by detergent. Additional evidence for the presence of aqueous spaces in the particles was obtained from investigation of their osmotic properties, which will be reported elsewhere.

Although ufasomes are less directly related to membrane lipids than liposomes prepared from natural phospholipids, they offer the enormous advantage for model membrane studies of chemical purity and simplicity. Pure fatty acids are relatively cheap, whereas the nearest approach to phospholipids containing specific fatty acids is through synthetic materials, which are either expensive or require fairly tedious organic procedures[8]. Compared with liposomes, ufasomes emphasize the hydrophobic component of natural membranes. But the many parallels we have observed in the structure and behaviour of both types of particles suggest that it is those hydrophobic components that are dominant in determining the properties of the membranes.

An interesting feature of ufasomes is the possibility that they can form from molecules synthesized in the absence of living organisms. Speculations on the origin of life have to postulate eventually the formation of compartments surrounded by membranes in which restricted surface permeability can allow the development of complex synthetic mechanisms[9]. There is good evidence that mixtures of long-chain hydrocarbons were formed by inorganic processes during the Cambrian and older eras. Analysis of hydrocarbons from ancient rocks showed the constituents to have a typical chain length of C-20 or greater and to be highly unsaturated[10]. No fatty acids or porphyrins were present in these mixtures.

In contrast, younger deposits associated with organic marine sediments contain mainly normal alkanes, saturated branch-chain compounds and some porphyrins and fatty acids. In a sequence of events leading to the formation of unsaturated fatty acids, some Cambrian hydrocarbon mixtures could be brought to the surface through earth movements and subsequently carboxylated by atmospheric carbon oxides[9]. Thin films of fatty acids could form in evaporates and it would only require a subsequent wetting at slightly alkaline *p*H and some wave action or ground tremor for the simplest known membranous particles, the ufasomes, to form. The particles might already contain molecules synthesized through the action of radiation on the constituents of the primitive atmosphere. Formation of amino acids, purines, and porphyrins under such conditions has been confirmed[11-13]. Alternatively, molecules such as NH_3, CO, CO_2, and CH_4 diffusing through the fatty acid membranes could condense within the particles in processes depending on a supply of external energy. Once formed, the more complex molecules would remain trapped for the lifetime of the particle. Other possibilities for synthesis of molecules essential for the appearance of life no doubt exist and are being actively studied in several laboratories. The feasibility of formation of ufasomes from simple prebiotic compounds may throw light on the mechanisms by which substances needed for appearance and development of living cells became concentrated and separated from their environment.

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Salicylates and Phospholipid Bilayer Membranes

FERREIRA[1] has observed that pain receptors are sensitized by prostaglandins, which strongly supports his suggestion that aspirin-like drugs induce analgesia by blocking the synthesis of prostaglandins. Salicylates, however, also produce an increase in the permeability of a molluscan neurone to potassium and a decrease in the permeability of the neurone to chloride which Barker and Levitan[2-4] have suggested may be related to the analgesic action of the drugs. More extensive pharmacological investigations are obviously required to ascertain whether analgesia could be a membrane related phenomenon, and these studies would be facilitated by a knowledge of the molecular mechanism by which salicylates modify the permeability of neurones to electrolytes. A simple working hypothesis is that the "receptor site" of the neuronal membrane for salicylate is the ubiquitous[5] phospholipid bilayer. An adsorption of salicylate anions to the bilayer would produce a negative electrostatic surface potential which would modify the interfacial ion concentrations and thus give rise to the observed changes in permeability. If the hypothesis is correct, salicylate should produce similar effects on neurones and artificial phospholipid bilayer membranes. It does.

Fig. 1 illustrates that salicylate increases the conductance due to the movement of a positive permeant species, the nonactin-K^+ complex, through a bilayer membrane formed from a decane solution of the amphoteric but neutral lipid, phosphatidyl ethanolamine (PE). It has been shown that the antibiotic nonactin forms 1:1 complexes with potassium[6], that it acts as a "neutral carrier" to transport the potassium ion across the membrane[7] and that the conductance it produces may be used to assess changes in the electrostatic potential at the surface of the membrane[8]. Specifically,

$$G^{\text{charged}}_{\text{Non-K}^+} = G^{\text{neutral}}_{\text{Non-K}^+} \exp -(F\psi/RT)$$

where $G^{\text{charged}}_{\text{Non-K}^+}$ is the conductance of the charged membrane, $G^{\text{neutral}}_{\text{Non-K}^+}$ is the conductance of the neutral membrane in the presence of the same concentrations of potassium and nonactin and ψ is the potential difference between the surfaces of the charged and neutral membranes ($RT/F = 25$ mV at 22° C). The data in Fig. 1*a* imply that salicylate has adsorbed to the surface of the bilayer and produced the potential indicated by the right hand ordinate.

The increase in conductance observed in Fig. 1*a* is not due to the permeation of salicylate itself because the salicylate conductance is very low at neutral *p*H (Fig. 1*b* and ref. 9) and the membrane can be shown to be potassium permselective. Furthermore, it is not due to a change in some parameter unrelated to the electric potential, such as dielectric constant or viscosity, because salicylate would then increase the conductance due to a negative permeant species. Fig. 1*b* illustrates, however, that salicylate decreases the conductance due to a negative permeant species, the complex formed between the undissociated acid and anion of 5,6-dichloro-2-trifluoromethylbenzimidazole (DTFB[10]). In particular, 10^{-1} M salicylate increases the cation conductance 1.5 log units (Fig. 1*a*) and decreases the anion conductance 1.2 log units (Fig. 1*b*). The changes in conductance are thus approximately equal in magnitude and opposite in direction, confirming that the main effect of salicylate is to produce a negative potential at the surface of the bilayer membrane.

Both charges and dipoles could contribute to the change in potential, but as similar effects are observed at *p*H 5 and 7 (*p*K salicylate = 3), the anionic rather than the undissociated form of salicylate must be responsible for the changes at neutral *p*H. As the theory of the diffuse double layer has been shown to describe adequately the potential due to charges at the surface of bilayer membranes[8,13–16], the Gouy equation may be used to estimate the charge densities required to produce the potentials illustrated in Fig. 1. These numbers agree qualitatively with direct equilibrium dialysis measurements[17]. It is therefore unnecessary to postulate that any substantial change in dipole potential occurs on adsorption of this particular molecule.

The degree of adsorption, moreover, does not appear to depend on the nature of the neutral lipid used to form the bilayer. When bilayers are formed from a 50% by weight mixture of cholesterol and phosphatidyl choline (PC) salicylate again increases the conductance due to a positive permeant species and decreases the conductance due to a negative permeant species (Fig. 2). Similar results have been obtained on bilayers formed from oxidized 7-dehydrocholesterol. The changes in conductance are approximately equal in magnitude, opposite in direction and identical with those observed in Fig. 1. It may be concluded, therefore, that the binding to these lipid bilayers involves primarily "hydrophobic" forces.

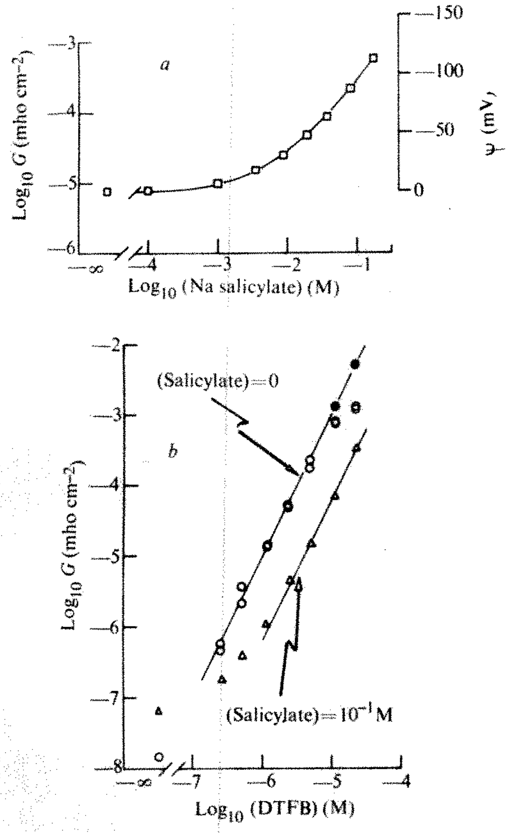

Fig. 1 *a*, Salicylate enhances the conductance of a cation selective bilayer membrane. The bilayer was formed from a decane solution of the neutral lipid PE in an aqueous solution of 10^{-1} M KCl, 10^{-6} M nonactin and either 2×10^{-3} M Tris or phosphate buffered to *p*H 7.0. The permeant species was the nonactin-K$^+$ complex. The right-hand ordinate illustrates the change in the electrostatic potential at the surface of the membrane calculated from the equation in the text. *b*, Salicylate depresses the conductance of an anion selective bilayer formed from PE. The bathing solutions contained 10^{-1} M KCl at *p*H 7.0 and the indicated concentration of sodium salicylate. The lines through the points have a slope of two, illustrating that the conductance, *G*, increases with the square of the DTFB concentration. This implies that the permeant species is a complex formed between the undissociated and anionic forms of DTFB, in agreement with results obtained with other substituted benzimidazoles[11]. The results of two experiments (○) are shown in one case to illustrate typical scatter in the data; all other points are the average of at least two experiments. ●, Instantaneous measurements when these differ from the steady state values because of diffusion polarization in the aqueous unstirred layers[12].

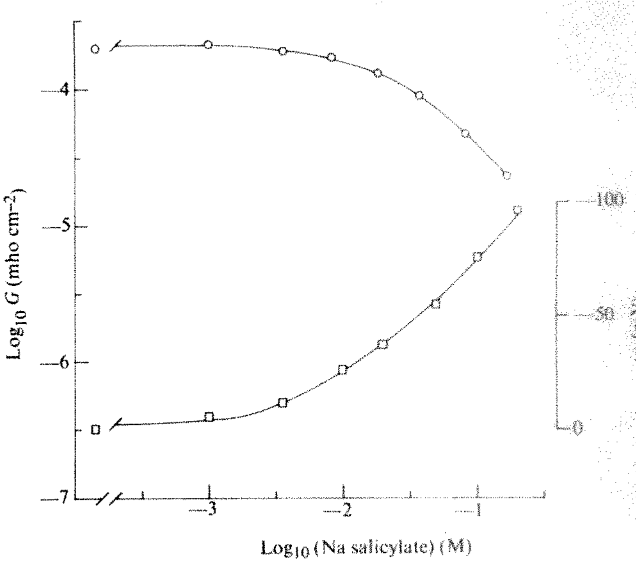

Fig. 2 Salicylate depresses the conductance due to a negative permeant species, ○——○, and enhances the conductance due to a positive permeant species, □——□, when the bilayer is formed from a 1 : 1 mixture of PC and cholesterol in decane. The bathing solutions contain 10^{-1} KCl, 2×10^{-3} M phosphate buffered to *p*H 7.0 and either 10^{-6} M nonactin (□) or 6×10^{-6} M DTFB (○).

The data obtained on these artificial membranes agree qualitatively with the results obtained on neurones from the mollusc *Navanax inermis*. On the neurones[2] salicylate, at concentrations from 3–30 mM, increased the potassium conductance. On the bilayers, it produced a measurable increase in the potassium conductance at a concentration of 3 mM and an order of magnitude increase at 30 mM. Also, for a large number of salicylate and benzoate analogues there is a good correlation between the octanol-water partition coefficient of the undissociated acid and the ability of the analogue to change the membrane properties of the molluscan neurone[18]. These observations support the hypothesis that salicylates produce their effects on neurones and bilayers in a similar manner because the relative degree to which the neutral form of the analogue partitions into an organic phase should correlate with the relative degree to which the anionic form of the analogue adsorbs to a bilayer. Thus, 3,5-dichlorosalicylate has a calculated partition coefficient into octanol of 50 times that of salicylate[18], is 30 times as potent as salicylate on a neurone[18], and produces effects on the conductance of PE and PC bilayers similar to those illustrated in Figs. 1 and 2, but is 100 times as potent as salicylate. Benzoate, conversely, has a lower octanol-water partition coefficient than salicylate and is less effective than salicylate in enhancing the potassium conductance of either a molluscan neurone[18] or a phospholipid bilayer.

A neutral analogue, salicylamide, also enhances the cation and depresses the anion conductance of neurones[4] and bilayer membranes at comparable concentrations, the bilayer conductances changing about an order of magnitude when the drug is present at a concentration of 10 mM. Identical effects are observed on the bilayers at *p*H 5 and 7 (*p*K salicylamide = 8.4) implying that the undissociated form of salicylamide is responsible for the changes in potential at neutral *p*H. The shapes of the curves obtained on bilayers are steeper than those illustrated in Figs. 1*a* and 2, as they should be, for this analogue must be changing the dipole[15,19] rather than the double layer potential at the membrane solution interface.

The recent observation[20] that a salicylate analogue, 5-bromosalicylate, changes the relative cation permeabilities of the *Navanax* neurone would seem to argue against the hypothesis that these drugs modify only the surface potential of the neuronal membrane. The permeability ratios, however, were calculated from the Goldman equation[21,22], which implicitly assumes that the surface potential remains constant on addition of the salicylate. In view of the fact that 2 mM 5-bromosalicylate produces a change of about -60 mV in the surface potential of PE bilayer membranes, it would seem more appropriate to calculate the permeability ratios from Frankenhaeuser's[23] modified form of the Goldman equation, which explicitly incorporates the effects of a surface potential on the concentrations of ions at the membrane solution interface. This equation indicates that salicylates do not significantly alter the permeability properties of the cation selective "channels"[24] in *Navanax*. All the effects of salicylates observed to date on phospholipid bilayers and *Navanax* neurones can be accounted for in terms of a simple molecular mechanism; the hydrophobic adsorption of the salicylate to the insulating bilayer and the concomitant production of a negative surface potential.

Salicylates and benzoates, furthermore, are not unique among the weak acids in their ability to adsorb to bilayer membranes. The uncoupler 2,4-dinitrophenol[25] (DNP), the fluorescent probe 1-anilino-8-naphthalene sulphonate[13] (ANS) and the indicator dye bromthymol blue (BTB) also adsorb: probably in much the same manner as salicylate because the shapes of the curves obtained with these molecules are all identical to that illustrated in Fig. 1*a*, but shifted about two, three, and four orders of magnitude to lower concentrations for DNP, ANS and BTB respectively. As "there is now substantial evidence that the major portion of the phospholipids is in bilayer form in a variety of intact membranes[5]", the anions of these and other amphipathic molecules should modify the surface potential of all essentially neutral biological membranes. Both squid axons[26] and frog myelinated nerves[27], however, seem to be negatively charged, illustrating the danger in extrapolating results obtained with amphipathic anions on either *Navanax* nerves or neutral bilayers to other systems.

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Synaptonemal Complexes in Brown Algae

THE small size of the nuclei of many brown algae makes it difficult to observe the chromosomes and their configurations in nuclear divisions, although life history studies and investigations of nuclei with the light microscope suggest that meiosis in these forms takes place in the unilocular sporangia. One means of testing the validity of this concept is to search for the presence of synaptonemal complexes because the relationship of meiotic chromosomes to these complexes has been well documented[1,2], and their presence has served as an indicator of meiosis in several members of the Myxomycetes[3,4] and for several species of red algae[5]. Our search for such complexes has been successful, and we describe here their occurrence in two species, *Chorda tomentosa* (Laminariales) and *Pylaiella littoralis* (Ectocarpales).

We collected material of both genera in April 1972 at Gloucester, Massachusetts. *Chorda* was fixed in 3% glutaraldehyde, *p*H 7.2, post-fixed in buffered osmium and embedded in epoxy resin[6]. *Pylaiella* was fixed in 1% glutaraldehyde in seawater, post-fixed in osmium (also in seawater) and embedded in 'Epon'. Sections of both plants were cut on a Reichert OM U2 ultramicrotome with glass knives, stained with either 2% aqueous $Ba(MnO_4)_2$ or lead citrate and viewed with a Zeiss EM 9A electron microscope.

Young sporangia of *C. tomentosa* undergoing their first

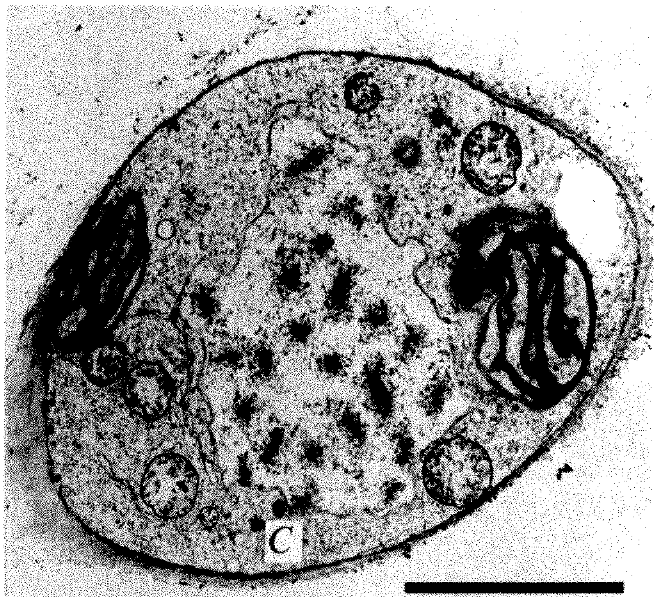

Fig. 1 Slightly oblique section of a young sporangium of *Chorda tomentosa* in prophase of meiosis. One of the two pairs of centrioles (*C*) can be seen near the nuclear envelope. Marker, 5 μm.

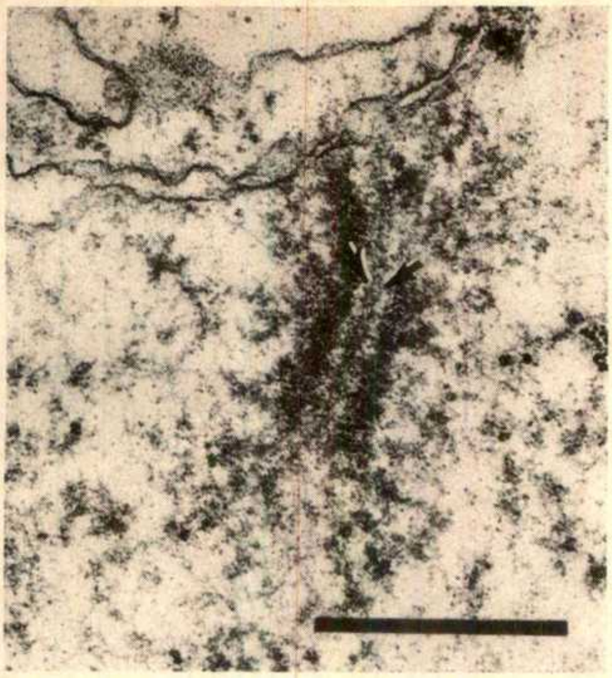

Fig. 2 Longitudinal section of a synaptonemal complex in *C. tomentosa*. The central element consists of two parallel bands (arrows). Marker, 0.5 μm.

nuclear divisions were observed in prophase of meiosis (Fig. 1), with numerous synapsed chromosomes present within the nuclear envelope. The synaptonemal complex in *C. tomentosa* (Fig. 2) shows the characteristic structure of three parallel electron-dense elements separated by less dense regions. The two side elements vary in width from 60 to 110 nm, and are separated by a 60–70 nm region containing the central element. The central element consists of two parallel narrow bands (arrows in Fig. 2).

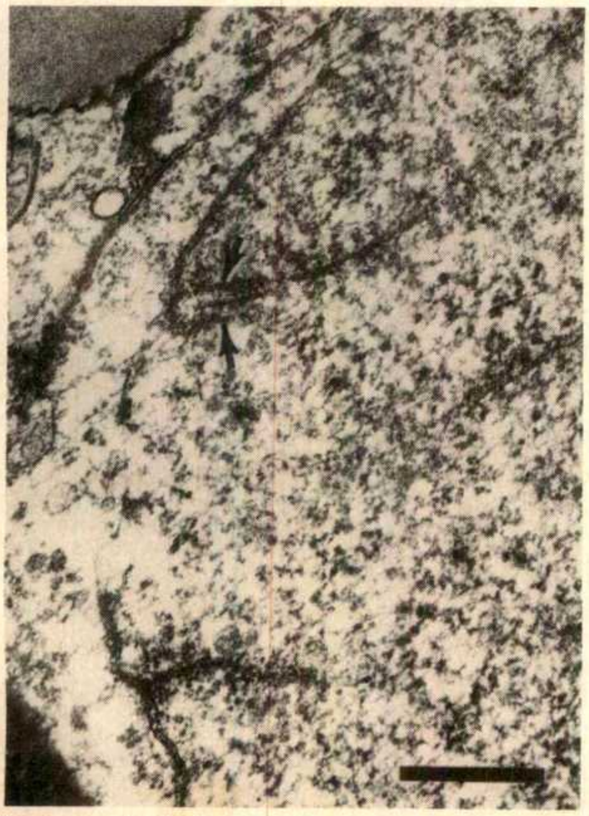

Fig. 3 Longitudinal section of a synaptonemal complex of *Pylaiella littoralis*. Arrows indicate the side elements. Marker, 0.5 μm.

Synaptonemal complexes in *Pylaiella littoralis* are found in nuclei of cells which are presumed to be developing unilocular sporangia. The synaptonemal complex consists of two side elements, 25–50 nm wide (arrows in Fig. 3), and a central element 25 nm wide (Fig. 3). The central space between the two side elements is approximately 50–75 nm wide. The synaptonemal complexes are close to the nuclear membranes (Fig. 3).

The discovery of synaptonemal complexes in *Chorda tomentosa* and *Pylaiella littoralis* helps to substantiate the concept that meiosis in the brown algae takes place in the unilocular sporangium. Serial sectioning and reconstruction using synaptonemal complexes[5,7,8] may also yield accurate chromosome counts for many algal species where the small nuclear size has prevented accurate counts from being made[9].

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Effect of Prohibition of the Use of Tetracyclines in Animal Feeds on Tetracycline Resistance of Faecal *E. coli* of Pigs

ALTHOUGH the use of tetracyclines in pig food in Britain was prohibited in March 1971 because of the accumulation of tetracycline-resistant *Escherichia coli* in the alimentary tract of pigs so fed, the veterinary use of tetracyclines continues. To assess the effect of this prohibition, I compared the incidence of these organisms in pigs brought to Chelmsford market in 1956, 1970 and July 1972.

On each occasion, thick aqueous suspensions of individual faecal specimens of 100 pigs, all of which had probably originated from different farms, were spread evenly over the surface of dried Petri plates of MacConkey agar and disks containing different antibiotics (Oxoid) were then applied at equal distances apart. The antibiotics were oxytetracycline (50 μg), streptomycin (25 μg), sulphonamides (300 μg), chloramphenicol (50 μg), ampicillin (25 μg), polymixin (300 units), spectinomycin (25 μg) and trimethiprim (1.25 μg), the actual antibiotics used depending on their availability. The plates were incubated at 37° C and read. The method of recording was subject to some variation, but in general it was as follows.

All sensitive—when a broad zone containing no colonies resembling *E. coli* surrounded a disk. Nearly all sensitive—when a few colonies resembling *E. coli* were present in the inhibitory zone around a disk. Resistant and sensitive—when a disk was surrounded by a mainly confluent band of *E. coli* growth, less profuse than the mass of the *E. coli* growth on the plate. All resistant—when growth of *E. coli* of uniform thickness took place up to the edge of a disk.

When difficulty was experienced in assessing sulphonamide and trimethiprim sensitivity, the relevant tests were repeated using DST Agar (Oxoid CM.261).

Table 1 The Antibiotic Sensitivity of the *Escherichia coli* in Faecal Specimens of Pigs Collected at Different Times from Chelmsford Market

Antibiotic	Year when specimens were collected	No. of 100 specimens in which the *E. coli* were: All resistant	Resistant and sensitive	Nearly all sensitive	All sensitive
Oxytetracycline	1956	18	13	22	47
	1970	64	22	7	7
	1972	36	41	16	7
Streptomycin	1956	0	0	2	98
	1970	15	45	20	20
	1972	7	42	30	21
Sulphonamides	1956	2	2	4	92
	1970	12	32	18	38
	1972	5	41	29	25
Chloramphenicol	1956	0	0	0	100
	1970	0	3	2	95
	1972	0	1	3	96
Ampicillin	1970	1	20	15	64
	1972	0	12	18	70
Neomycin	1970	1	1	5	93
	1972	0	1	1	98
Furazolidone	1970	1	3	4	92
	1972	1	1	6	92
Polymixin	1970	0	0	0	100
	1972	0	0	0	100
Spectinomycin	1972	3	20	26	51
Trimethiprim	1972	0	0	0	100

The results are summarized in the table. Employing the method of Smith[1], the tetracycline resistance of 40 of 60 *E. coli* strains isolated from different faecal specimens in the 1970 and 1972 surveys was transmitted to a nalidixic acid-resistant mutant of *E. coli* K12F$^-$. Of 30 streptomycin-resistant strains isolated from different pigs in the 1972 survey, 28 were resistant to sulphonamides and 16 to tetracyclines; the resistance of 13 of 28 of the strains resistant to streptomycin and sulphonamides was transmitted to the K12F$^-$ strain.

It is probable that the substantial amount of tetracycline-resistant *E. coli* found in the 1956 survey was mainly the result of the first few years of use of tetracyclines as feed additives and the much greater amount in the 1970 survey the result of their continued use. If these results are typical of the position in Britain as a whole, then in 1970 the dominant *E. coli* organisms in the pig population were tetracycline-resistant ones, usually possessing conjugation ability and, as such, potentially able to transmit plasmids other than those coding for tetracycline-resistance. The 1972 survey suggests that during a 16 month period of prohibition of the use of tetracyclines as feed additives, the incidence of pigs harbouring tetracycline-resistant *E. coli* in their faeces had not decreased but that the amount of tetracycline-resistant *E. coli* in the pig population might have. The greater incidence of *E. coli* resistant to streptomycin and sulphonamides in the 1970 and 1972 surveys must be regarded mainly as the consequence of the therapeutic use of these drugs because they are not employed as feed additives for pigs.

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Supercontracting Striated Muscle in a Vertebrate

THE capacity of vertebrate striated muscle fibres to shorten is much curtailed by their Z-disks. Skeletal muscle sarcomeres, for example, can be reversibly contracted to about 40% of their fully extended length[1], an observation that accords well with the sliding filament model of muscle contraction[2,3]. Once the thick filaments slide up against the Z-disks at the ends of the sarcomere, further shortening merely causes crumpling of the ends of the thick filaments[1] and a rapid decrease in tension. In marked contrast many unstriated muscles can exert tension whilst shortening to less than 10% of their fully extended length, as they have no Z-disks to interrupt the sliding of their filaments[4]. The regimenting and compartmentalizing influence of Z-disks in striated muscle supports speed and power of contraction but it is at the expense of shortening capacity.

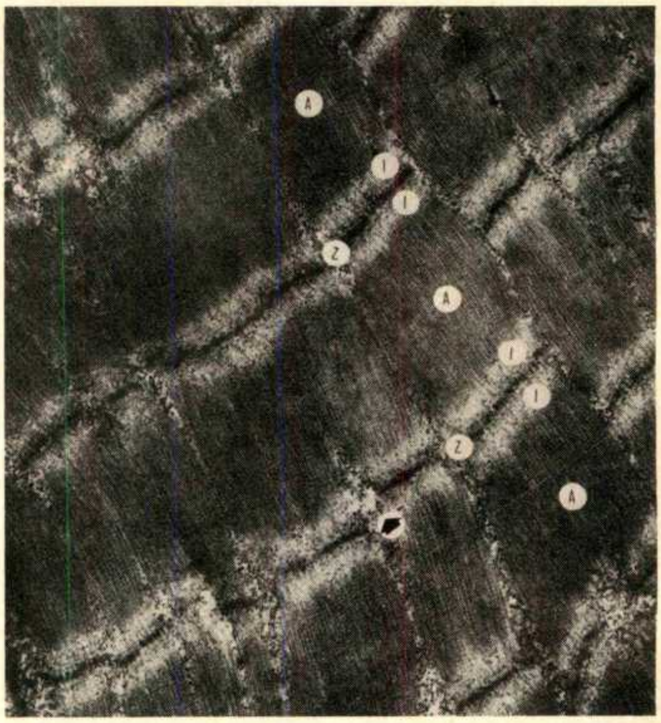

Fig. 1 Longitudinal section of chameleon tongue retractor striated muscle fibre ×13,200. Z, Z-disks; A, A-zones; I, I-zones; arrow, glycogen granules.

In view of these well established properties of vertebrate striated muscle it was surprising to find striated muscle fibres in the tongue retractors of the chameleon (Fig. 1). This animal can project its tongue a considerable distance when attempting to capture prey and, in returning the extended tongue to the mouth, the retractor muscles shorten considerably and at high speed. The striated fibres concerned are longitudinally orientated, running from their origin on the hyoid to insert on the inner surface of the lingual bulb. Measurement of the resting and extended lengths of the tongue retractor muscle fibres showed a remarkable shortening capacity (Table 1).

Table 1 Shortening Capacity of Chameleon Tongue Muscles

Animal No.	Retracted length R (mm)	Extended length E (mm)	Ratio $R:E$
1	30	184	1 : 6.1
2	28	178	1 : 6.4
3	30	190	1 : 6.3
4	22	136	1 : 6.2

By electrically stimulating the retractor muscles it was found that they can powerfully and repeatedly contract down to about 16% of their extended length. This is a much greater shortening capacity than any previously described in vertebrate striated muscles and can be termed reversible supercontraction from analogy with certain invertebrate muscles[5].

As it is the Z-disks that impose shortening limitations on most vertebrate striated muscles, these were examined for specialization. I decapitated chameleons, extended their tongues to about half their full length and prepared them for electron microscopy, taking care to maintain isosmotic conditions. At low magnification the tongue retractor muscle fibres showed the classical striation pattern, sarcomeres with A and I-zones and Z-disks being clearly demarcated; H-zones were sometimes evident although M-disks were not identified. Higher magnifications showed each sarcomere to have an almost conventional striated muscle arrangement, with thin filaments (diameter 5 nm) in the I-zone and thick filaments (diameter 20 nm) restricted to the A-zone. Regular perpendicular bridges, periodicity 35 nm, linked the two types of filaments together in the region of overlap; these resemble the cross-bridges found in vertebrate and insect muscle[6,7], although in the chameleon muscle the periodicity sometimes seems to extend into the I-zone and may represent troponin sites.

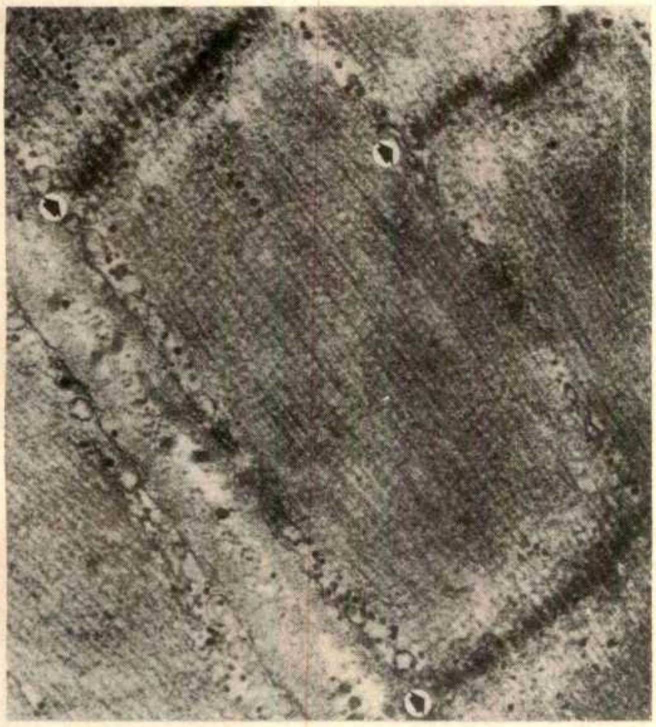

Fig. 2 Longitudinal section of chameleon tongue muscle sarcomere, ×36,080. Arrows, perforated Z-disks.

The Z-disks have a very unusual appearance; instead of the normal continuous structures which completely separate the sarcomeres, they are discontinuous, presenting a fragmented appearance in longitudinal section (Figs. 2–3) and a shredded appearance in transverse section (Fig. 4). Three-dimensional reconstruction of the Z-disks of chameleon tongue muscle sarcomeres reveals that they are each divided into about twenty strips, each 30 nm wide; thin filaments from each side are attached to the strips in alternate pairs. The slits between the strips are open, enabling adjacent sarcomeres to communicate. The ratio of the numbers of thin filaments to thick filaments is approximately 4 to 1, much higher than in other vertebrate striated muscles.

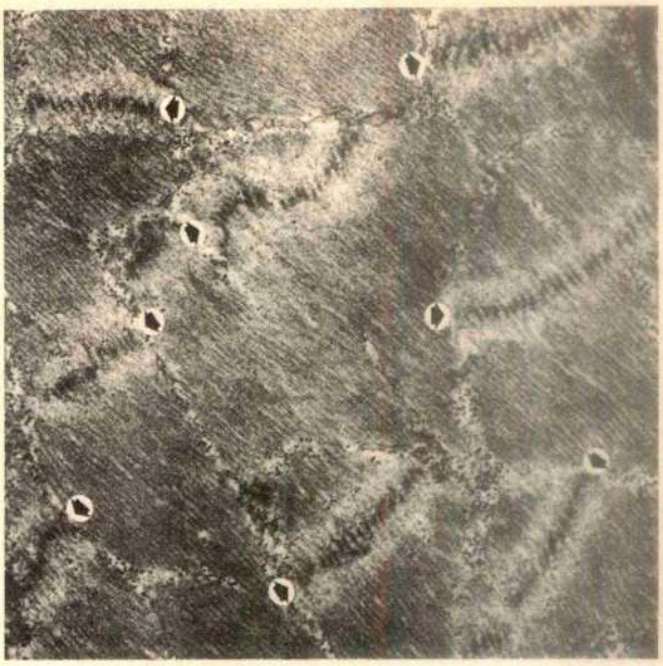

Fig. 3 Oblique section of chameleon tongue muscle, ×16,400. Arrows, perforated Z-disks.

Supercontracting striated muscles occur widely among the arthropod invertebrates and were first described in a barnacle[8]. A second report concerned the insects—the body wall muscles of the blowfly maggot were found to supercontract[9]. Insect visceral muscles are all striated and many of them are of the supercontracting type[10]. As a result of these invertebrate studies (especially ref. 9), the process of reversible supercontraction is well understood in terms of the sliding filament model[2,3].

The mechanism of excessive shortening is based on the continuity of the sarcomeres by way of the perforations in their Z-disks. Contraction is not curtailed when thick filaments abut on Z-disks; instead the filaments slide through the holes into adjacent sarcomeres and shortening proceeds with little decrease in tension. By this process supercon-

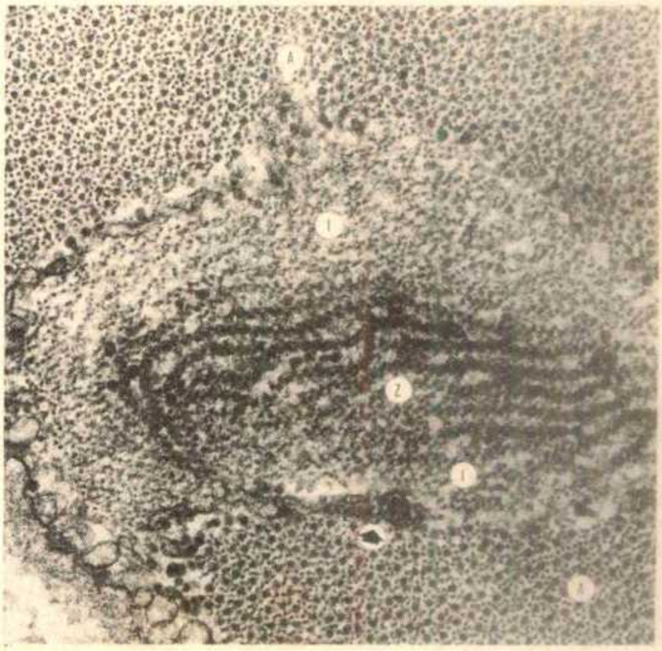

Fig. 4 Transverse section of chameleon tongue muscle, ×41,000. Z, Z-disk; A, A-zones; I, I-zones; arrow, triad.

tracting muscles in arthropods combine the properties of striated and unstriated muscles[10]. In the chameleon, too, the striated tongue retractor muscle fibres have much of the force and speed of skeletal muscle (preliminary observations give a maximum tension greater than 1 kg cm^{-2} and a maximum velocity greater than 1 m s^{-1}) in conjunction with a shortening capacity normally available only to unstriated muscle. The Z-disk perforations in chameleon muscles differ, however, from those of arthropod muscles, suggesting independent evolution of the supercontracting habit. A more detailed account of chameleon tongue muscles will be published elsewhere.

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Effect of Cigarette Smoking on Primary and Secondary Humoral Responses of Mice

THE bronchopulmonary system, one of the most important sites of access for antigen into the body, has a highly developed lymphatic system[1] and the capacity to effect a local immune response[2]. Any modification of these defences by the environment is likely to have a lasting influence on the health of an animal. Chronic exposure to sulphur dioxide and carbon has been found to decrease the immune response of mice to inhaled antigen[3]. Cigarette smokers have also been found to have decreased serum antibody to influenza virus after infection or vaccination[4]. Both air pollution and cigarette smoking have been associated with an increased susceptibility to respiratory infection[5,6].

Antibody-producing cells have been found in the lungs of experimental animals after intratracheal (IT), intravenous (ref. 7 and our unpublished results) and intraperitoneal[8] inoculation of sheep erythrocytes (SRBC). After IT inoculation antibody-producing cells have also been found in the spleen, blood and mediastinal lymph nodes (ref. 7 and our unpublished results). We have used the Cunningham modification of the Jerne plaque technique[9] to locate direct (IgM) and indirect (IgG/IgA) plaque forming cells (p.f.c.) in smoked and unsmoked mice after IT inoculation of SRBC. Strictly line bred C57Bl mice were exposed to smoke for 26 weeks in a machine[10] set to give a 1/7 smoke/air ratio at a puff volume of 35 ml. The animals were exposed to the smoke of thirty cigarettes daily (king size filter supplied by the Australian Tobacco Research Foundation). Animals were inoculated IT with 10^8 SRBC in 10 µl of phosphate buffered saline (PBS) and the smoking regime continued. A second inoculation was given on day 12. Groups of 5 mice were examined for antibody producing cells at 7, 9, 12, 15, 18 and 21 d after the first inoculation. The animals were exsanguinated and the blood leukocytes purified by dextran sedimentation. The cervical and mediastinal lymph nodes, spleen and lungs were removed, washed in PBS, minced, filtered through nylon wool and then washed and resuspended in MEM. About 10^6, 10^7 and 10^8 white blood cells were recovered from the lung, lymph nodes and spleen respectively.

After IT inoculation the principal site of antibody synthesis in normal and smoked animals is the spleen, followed by the lymph nodes, with a small response in the lungs. The results are similar when considered per organ or per 10^6 leukocytes. The only significant response found in the blood was in unsmoked mice 6 d after inoculation (5±2 direct p.f.c./10^6 leukocytes). Responses in the spleen, lymph nodes and lung for smoked and unsmoked mice are shown in Fig. 1. All organs of the unsmoked, age matched controls developed a primary response, and after a second inoculation show an anamnestic response with increased indirect p.f.c.

Cigarette smoke modifies the immune response in all organs. The primary response in the lungs is virtually abrogated and

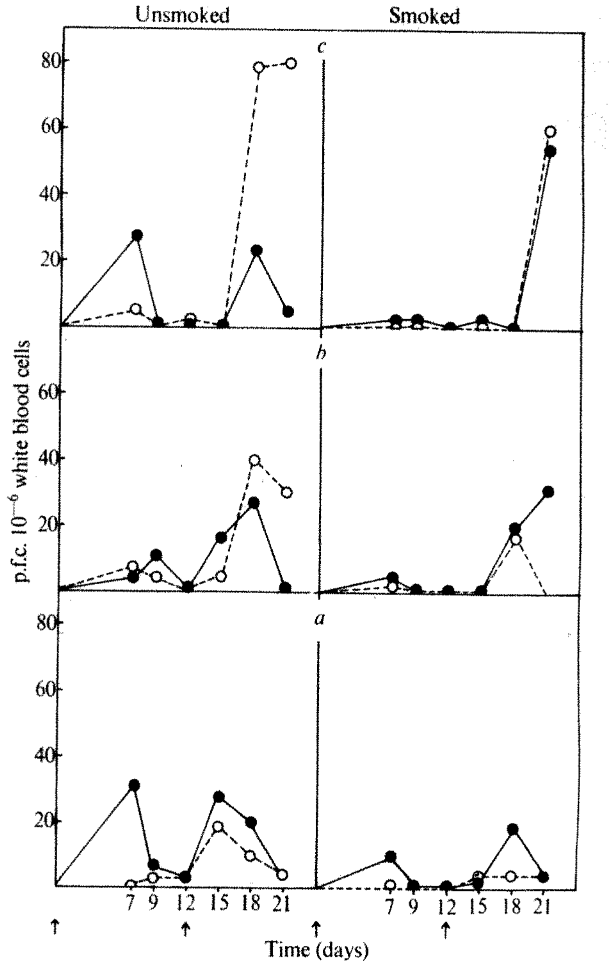

Fig. 1 Haemolytic plaque forming cells in (*a*) the spleen, (*b*) lymph nodes and (*c*) lungs of smoked and control mice after IT inoculation of SRBC. The smoked mice were exposed once daily (8 min) for 26 weeks in a Hamburg II small-animal smoking machine (Heinr. Borgwaldt, West Germany) set to deliver a 1 : 7 smoke : air mixture. All mice received a primary IT inoculation of 10^8 SRBC on day 0, and a secondary IT challenge on day 12 (inoculation times indicated by arrows). Lung cells were suspended in Eagle's MEM at approximately 5×10^6 ml^{-1}, spleen at 5×10^7 ml^{-1} and lymph nodes at 5×10^7 ml^{-1}. 0.02 ml of 8% SRBC, 0.02 ml of guinea-pig complement and either 0.02 ml of PBS (for direct plaque) or 0.02 ml of a suitable dilution of anti-mouse globulin antiserum (for indirect plaques), was added to 0.02 ml of the cell suspension. The reaction mixtures were placed in Cunningham chambers in duplicate and sealed with wax. The plaques were counted after 30 min incubation at 37° C. ●—●, Direct p.f.c.; ○ - - - ○, indirect p.f.c.

although it is initiated in the spleen and lymph nodes it is reduced and short lived. After a second inoculation the response in the spleen of smoked animals was again reduced (particularly the indirect p.f.c.). The secondary response in the lungs is delayed longer than a normal primary response but resembles a secondary response in magnitude but with a greater participation of direct p.f.c. We found no acceleration of the secondary response in the lymph nodes, and although there is some increase in both direct and indirect p.f.c. the direct p.f.c. predominates.

The degree of reduction of the response seems to be related to the distance of the organ from the primary point of entry of the cigarette smoke. The immunosuppression recorded may well explain the increased susceptibility of smokers to respiratory infections. If immune surveillance[11] has a role in the elimination of cancer cells, then irrespective of any carcinogenic activity of cigarette smoke, damage to lung immunity may allow the escape of the cancer cell from a basic control mechanism.

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State-dependent Effects of Marihuana on Human Memory

THE abuse potential of marihuana has been widely debated as the use of the drug has increased in Western countries. Overton[1] has suggested that the abuse potential of various drugs may parallel their capacity for producing "state-dependent" effects in animals and man. State-dependent or "dissociated" learning refers to a phenomenon observed in animal and human studies wherein certain behaviour learned in particular drug states transfers more readily when the same drug state is reintroduced than if subsequent testing occurs under the influence of another drug or in a non-drug state[2–8]. This phenomenon has been demonstrated primarily with sedative and hypnotic drugs, such as barbiturates and alcohol, which are relatively susceptible to abuse. In general, drugs with little abuse potential (for example phenothiazines) dissociate weakly or not at all[1].

Barry and Kubena[9] have shown that Δ^9-transtetrahydrocannabinol produces state-dependent effects in rats. In the study reported here we examined the effects of smoking marihuana on various tasks, to see whether the drug produces state-dependency in man. If Overton's hypothesis is correct, detection of state-dependent effects from marihuana would be associated with its possible abuse potential.

Thirty-two men between the ages of 21 and 30 were paid to participate in a training session (day 1) and a test session (day 2) separated by 24 h. All subjects were screened for inclusion in the study using a structured medical and psychiatric interview. No subjects showed evidence of medical or psychiatric illness. All were experienced marihuana smokers; more than half had smoked during 4 or more years at least once a week. None had used marihuana or other drugs for at least 48 h before the experiment. The study was run single blind; that is, the subjects were not told that a placebo would be given. In most cases subjects given placebo were unaware they were receiving it. Because of the objective nature of the tasks, it was not necessary that experimenters be unaware of what subjects received.

Subjects were randomly assigned to one of four groups. One group (MM) received marihuana on both days. A second group (PP) received marihuana placebo on both days. A third group (MP) received marihuana on day 1 and placebo on day 2. A fourth group (PM) received placebo on day 1 and marihuana on day 2. This factorial design permitted analysis of drug, day and change-state effects. The change of state effect (*F* value) is reported in Table 1.

Table 1 Mean Errors and *F* Values for Change of State

Task	Day 2 – mean errors				*F* value
	PP	PM	MP	MM	
Ordered object recall	0.25	3.13	2.88	1.13	12.46*
Avoidance	14.25	13.88	15.38	10.88	0.64
Word association	0.50	2.50	1.25	3.13	0.01
Word strings					
Type *a*	0.69	1.75	1.32	0.94	1.00
Type *b*	2.19	3.07	2.19	2.37	0.15
Type *c*	1.94	2.75	2.62	2.00	0.63
Type *d*	3.06	4.82	6.19	3.94	5.78†
Types *a–d* (combined)	20.50	17.56	20.06	20.00	1.00

Data are based on analysis of four groups of eight subjects each. The first letter refers to the condition on day 1 and the second letter refers to the condition on day 2.

* $P > 0.01$.
† $P > 0.05$.

The marihuana contained 1.5% (−)-Δ^9-transtetrahydrocannabinol (THC) whereas the placebo consisted of marihuana with THC removed. Both drug and placebo were obtained from the National Institute of Mental Health. The marihuana was equivalent in strength to "street" marihuana imported into the United States from Mexico.

The procedure used in combusting and administering the smoke was similar to that used by Renault *et al.*[10]. A 9 l. spirometer (Collins Spirometer) with attached crucible was used. A 1 g sample of placebo or marihuana was burned in the crucible by pulling air through the material as the inner bell of the spirometer was raised, trapping the smoke in the bell. The crucible was then removed and replaced by a rubber mouthpiece. Subjects were instructed in the method of breathing to be used, consisting of inhalation for 5 s, holding the breath for 15 s, and resuming normal breathing for 35 s. This sequence was repeated until three canisters of smoke were inhaled; this required about 15 min. Testing began 15 min after smoking finished.

Tests included a visual avoidance task; a word association recall task; a verbal learning task measuring memory for "word strings" of varying degrees of meaningfulness, and a test measuring recall of ordered objects. Tests were given in a counter-balanced order during a period of 1 h. All tests except the visual avoidance task were designed to measure positive transfer of learning from day 1 to day 2. The avoidance task measured interference or negative transfer. Where positive transfer is measured, dissociative learning is suggested when mean error scores in the same-state groups are lower than in the change-state groups. In the avoidance task, designed to measure negative transfer, dissociation would be reflected by fewer errors in the change-state groups than in the same-state groups.

In the avoidance task, four patterns of lights were randomly presented. Each pattern could be extinguished by a specific switch controlled by right and left hands and feet. An incorrect response or failure to respond within 3 s resulted in presentation of a noxious tone. The criterion was twenty correct responses, with number of errors to reach criterion taken as the measure of performance. The task was identical on both days, except that on day 2 the pattern-switch relation was altered. Thus, performance on day 2 was assumed to reflect interference; that is, the greater the number of errors on day 2, the greater the degree of interference.

The rote learning task involved memorizing four five-word "word strings" of varying meaningfulness (normal sentence, anomalous sentence, anagram and word list)[11]. On day 2 subjects were asked to recall the word strings memorized on day 1 after which a relearning session was conducted. The following word strings were used. (*a*) The normal sentence was: "Soapy detergents dissolve greasy stains"; (*b*) the anomalous sentence was: "Pink accidents cause gallant storms"; (*c*) the anagram was: "Neighbours sleeping noisy wake parties"; (*d*) the nonsense word list was: "give odours snows fatal violent". Performance was measured in terms of errors of sequence and omission.

For the word association test, twenty words of low association value[12] were presented. Subjects were instructed to respond to the stimulus words with the first word that came to mind. On day 2 the stimulus words were repeated and subjects were asked to recall their responses on day 1. Performance was measured in terms of errors made in day 2 recall.

In the object recall test subjects were shown seven plastic objects from the Kahn test of symbol arrangement[13]. They were asked to name each so that subsequent deficits in recall presumably would reflect memory loss rather than inattention. The subjects were then asked to order the objects from left to right. On day 2 subjects were asked to recall the seven objects and then arrange them in the same order as on day 1.

All measures of performance were subjected to 2 by 2 factorial analyses of variance. Table 1 presents the interaction F values for the four tasks and the mean errors for each group. The interaction effect tests the marihuana state change effect. Thus, any significant interaction may be taken as evidence of dissociation.

As Table 1 shows, the recall of ordered objects showed a significant interaction effect ($P<0.01$). The dissociative effect arose from the MP and the PM groups making more errors than either the PP or MM groups. Analysis of simple principal effects indicated that both the PM and MP groups differed significantly from the PP group ($F=9.63$, $P<0.01$; and $F=8.03$, $P<0.01$, respectively). Also, the PM group differed significantly from the MM group ($F=4.66$, $P<0.05$).

Neither the avoidance task nor the word association task revealed a significant interaction effect. In the word string task, with errors analysed by type of string, there was evidence of dissociation for the type *d* string (Table 1) in which both syntax and meaningfulness are missing. The other three types of word strings provided these cues. The normal sentence provided both syntax and meaningfulness, while the anomalous sentence provided syntax, and the anagram provided meaningfulness

Although an interaction effect was not found in the word association task, a significant drug effect was evident ($F=8.73$, $P<0.01$). Thus, marihuana disrupts memory for self-generated word associations, though this effect is no more pronounced in the change-state groups than in the same-state groups.

The significant results found in the ordered object recall and the type *d* word string (Table 1) suggest that tasks involving sequential memory may be most sensitive to dissociation. The lack of dissociation found for the *a*, *b*, and *c* type word strings may be explained by the fact that recall of the sequence is aided by long term memory for sentence structure or meaningfulness of words. In contrast, the type *d* string must be recalled sequentially without the aid of either. This task seems to have much in common with sequential recall of the plastic Kahn objects, where meaningfulness also plays an insignificant role.

The results of the present study suggest that marihuana produces "state-dependent" learning when tasks sensitive to dissociation are used. In other studies of state-dependent learning, using other drugs, similar differential effects were found, depending on the task and the drug administered. Other investigators have reported disruptive effects of marihuana on tasks involving sequential adjustments in reaching a goal[14].

If the abuse potential of drugs parallels their capacity to produce state-dependent or dissociated learning, as Overton[1] has suggested, then one must conclude that marihuana has an abuse potential. However, the degree of dissociation obviously varies as a function of task variables in addition to any correlation it may show with abuse potential.

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Survival of Common Bacteria in Liquid Culture under Carbon Dioxide at High Temperatures

WE report results which are at variance with the general belief that high temperatures are necessarily lethal to microorganisms[1]. *Escherichia coil* B, *Aerobacter aerogenes*, *Bacillus subtilis*, and *Serratia marcescens* were found to survive for 24 h in a CO_2 atmosphere at a temperature of up to 160° C. These organisms were routinely cultured in sterile trypticase soy broth (TSB) medium, and plated on TSB plates containing 1.5% of agar.

Table 1 Effect of Temperature and High Pressure CO_2 on the Survival of *E. coli*, *A. aerogenes*, *S. marcescens*, and *B. subtilis*

Expt	pCO_2* (atm)	Total pressure †	Time (h)	Temp. (°C)	Viable counts‡ Control Final	Viable counts‡ Exposed	Survival (%)
E. coli							
1	10.1	11.2	24	105	3.6	7.3×10^{5}	2.0×10^{-3}
2	10.1	11.2	19	110	8.6	3.1	36
3	11.3	12.7	24	110	4.2	1.0×10^{-4}	2.4×10^{-3}
4	9.5	10.9	24	110	0.5	1.0×10^{-3}	0.2
5	9.5	10.9	24	110	3.4	2.5×10^{-3}	7.0×10^{-2}
6	9.5	10.9	24	110	3.7	1.9×10^{-5}	5.0×10^{-4}
7	9.5	11.8	24	125	4.0	4.0×10^{-4}	10^{-2}
8	9.5	11.8	24	125	3.4	2.0×10^{-5}	6.0×10^{-4}
9	9.5	11.8	24	125	3.8	1.3×10^{-3}	4.0×10^{-2}
10	9.5	11.8	24	125	2.7	2.2	81
11	17.0	20.3	20	125	11.5	3.0	26
A. aerogenes							
12	9.5	11.8	24	125	5.4	0.25	4.7
13	9.5	11.8	24	125	5.4	0.27	5.0
14	19.7	25.8	24	160	1.3	5.1×10^{-4}	4×10^{-2}
15	19.7	25.8	24	160	0.36	2.9×10^{-4}	8×10^{-2}
16	19.7	25.8	24	160	0.27	10^{-6}	4×10^{-4}
17	19.7	25.8	24	160	0.29	8.0×10^{-5}	3×10^{-2}
18	19.7	25.8	24	160	0.34	0	0
19	19.7	25.8	24	160	0.31	4.3×10^{-3}	1.4
20	19.7	25.8	24	160	0.32	5.0×10^{-5}	1.6×10^{-2}
S. marcescens							
21	10.1	11.2	19	105	1.6	0.02	1.2
22	11.3	12.7	24	110	5.3	1.1×10^{-4}	2.1×10^{-3}
23	11.3	12.7	24	110	6.9	1.3×10^{-4}	2.0×10^{-3}
24	9.5	11.8	24	125	5.2	1.1×10^{-3}	2.0×10^{-3}
25	18.0	22.9	21	152	2.8	6×10^{-6}	2.1×10^{-4}
26	19.7	25.8	4.5	160	14.0	5.0	36
B. subtilis							
26	9.5	11.8	24	125	3.3	0.21	6.4
27	19.7	25.8	4.5	160	4.2	5.4×10^{-6}	1.3×10^{-4}

* Given at the experimental temperature. † Including water vapour pressure, at the experimental temperature[13]. ‡ Figures have been multiplied by 10^8, and represent cells per ml. given as exposed/control counts (viable, after 24 h at 20° C), expressed as a percentage.

Cultures were incubated at 37° C on a rotary shaker, subcultured every 48 h, and exposed to high temperatures and pressures while in the stationary phase, 48 h after subculturing. One millilitre of each culture was injected into 5 ml. glass tubes, which were then inserted into 75 m stainless steel bombs fitted with a plug and a needle valve, and flushed free of air with commercial grade CO_2 for 30 s before sealing and raising the pressure to up to 20 atm, and enclosing the entire bomb in an oven at 105–160° C for up to 24 h. Pyrene (m.p. 150° C) was liquefied inside such a bomb within 1 h at an oven temperature reading of 150° C, demonstrating effective heat transfer. Control tubes of each culture were maintained at 20° C in air during the experiment, to provide data on viability loss through standing.

After exposure, dilutions of 10^1–10^8 were made of test and control cultures, by transferring 1 ml. of each into 9 ml. of sterile TSB in screwcapped tubes, shaking, and repeating the transfer eight times, using a new 1 ml. disposable pipette for each transfer. Each dilution was then plated in triplicate and incubated at 37° C for 7 days, when viable counts were obtained.

As checks on possible contamination, we used (1) microscopic examination of exposed cultures, (2) a procedural blank of 105 plates obtained from a sample of medium exposed for 24 h at 105° C and 9.5 atm CO_2 inside a bomb, and (3) the Enterotube diagnostic method[2]. The control experiment was performed throughout as though a live culture were being used, and yielded four plates contaminated with fifteen colonies, which were easily distinguished from our test organisms, and which appeared after incubation for 6–7 days on the top plates of the stacks. The Enterotube test (Roche Diagnostics, Nutley, NJ) consisted of the inoculation of colonies obtained on plates from previously heated and pressurized cultures, into the Enterotube, which was then incubated at 37° C for 24 h. These tubes give colour changes which are diagnostic for individual species. In fourteen of fifteen colonies of *E. coli* and *S. marcescens* which were thus tested after exposure to high temperature and pressure, the result was positive for the test organism.

The results obtained are summarized in Table 1. There were, however, six experiments, of which three were with *E. coli*, two with *B. subtilis* and one with *A. aerogenes*, in which no survival was recorded. The percentage survival varied from 0 to 81.

The medium, after exposure, was clear yellow with no sign of charring. Cells were mobile when viewed under the microscope, although some clumping was noted. The motion was not Brownian motion.

The composition of the gas phase inside the bombs was not constant during an experiment, or between experiments, as different corrections had to be applied for the saturation vapour pressure of water. At 160° C this correction is 6.1 atm. Simple calculation shows that the water loss never exceeds 18% of the total volume. The cultures are therefore never dry. The carbon dioxide gas used was analysed by mass spectrometry and had the following composition: H_2O (11 p.p.m.), Ar (1), CH_4 (1), N_2 (24), CO (<1) and O_2 (225).

These results cannot be explained as contamination. Our controls from the Enterotube results were positive. Further *S. marcescens* yielded red colonies after exposure to high temperatures and pressures. As we are unable to detect a false step in our experimental procedure which could have led to these results, we believe the data are real. Common microorganisms thus appear to be able to survive long periods at temperatures normally considered lethal, if carbon dioxide is present. These results may have some implications for the exploration of the atmosphere of Venus by the use of space probes. Sterilization of space probes may be necessary.

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Visibility of Vehicle Rear Lights in Fog

While driving in daylight fog vehicle rear lights often become lost to view at about the same distance as the vehicle carrying them. The brighter the light, the further will it be visible through fog[1,2]. That the area of the light itself is also important is less well known.

A ray of image-forming light, in its passage from an object to the eye, is reduced in intensity by absorption and by light being scattered out of its path by the water droplets in the fog. There is, simultaneously, a gain in brightness as a result of non-image forming diffuse light producing a veil of brightness between the eye and the object. Thus the apparent luminance of both the vehicle and its lights tends to equal that of the background fog. As the viewing distance is increased the contrast between the vehicle and its lights diminishes to a level that the eye cannot detect.

Contrast can be defined[3] as $(O-B)/B$ where O is the object radiance and B is the radiance of its background. For monochromatic light Duntley[4], using Koshmieder's equation[5] for radiance transfer through turbid media, has shown that the apparent contrast between an object and its background decreases exponentially with distance. If O is a light source, the brighter the light the further away will it be visible. Accurate calculations must take into account the sensitivity of the eye, the radiance distribution of O and B and the spectral transmission of the intervening medium. For most field work on visibility, however, these factors, including the red colour of the light, are not very significant[6].

The intensity of the ambient light and the angle subtended by the object at the eye are the principal factors affecting the ability of the eye to detect contrasts[3]. In daylight fog the intensity of the ambient light is high, but so is the veiling light. Thus visual contrasts tend to be low, but the contrast perception threshold is also low. Above about 1° the visual angle is not very significant, but for smaller angles the contrast perception threshold of the eye increases rapidly. A light 6 cm in diameter subtends a 1° angle at 3.4 m so in most traffic situations the angular subtense of the rear light is significant.

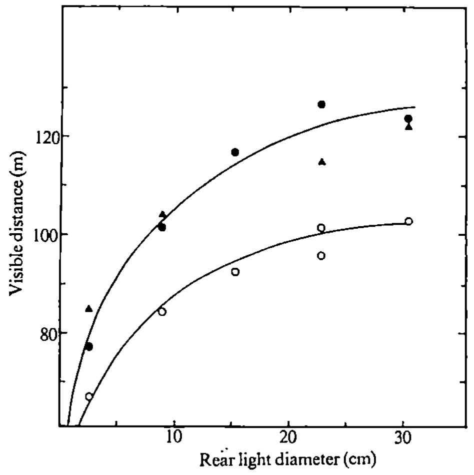

Fig. 1 Distance that red lights of various diameters can just be distinguished through fog by the two observers, J. N. L. (○, ●) and E. R. C. (▲). ○, 1000–1030 h on January 23, 1973; ●, ▲, 1040–1105 h on January 25, 1973.

Fig. 1 shows the visible ranges of red lights of various diameters in hill fog on Ditchling Beacon, Sussex, England. A uniform red light 30.5 cm in diameter was obtained by mounting a Fresnel filter and a red acetate filter in front of a 48 W tungsten headlight 18 cm in diameter. The light was diffused and dispersed by two sheets of clear glass between the lamp and the lens. The first sheet had a stippled pattern, the second a diamond pattern. The diamond glass was in the plane of focus of the lens. Lights of different diameter were obtained by a series of black masks.

The light had an intensity of 3.5 lm cm^{-2}, which is roughly equivalent to an average brake indicator light or one of the less bright purpose-made accessory rear fog lamps[2]. The light was mounted in the rear of a red 22 cwt Bedford van. A cord was extended from the rear of the van away from the light. The observers walked back along the cord until the point at which the light could just be distinguished. The position on the cord was then marked with tape. Immediately afterwards a second measurement was taken for the visible range of the black surround to the light. There was no measurable difference between the visibility of the black surround and the red van.

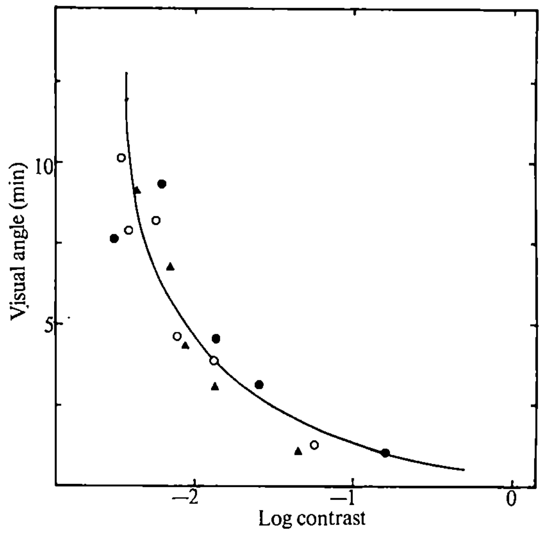

Fig. 2 Relationship between the visual angle (arc min) subtended by the test lights, and the calculated brightness contrast at which they are just perceptible. The points correspond to those in Fig. 1. By comparison, the curve is drawn from Blackwell's data[3] for the contrast perception threshold for different visual angles in good light (1,000 foot L).

There was no overall change in the density of the fog during the course of the experiments, although on both occasions a second experimental series had to be abandoned when the fog began to lift rapidly. The estimates for the visible range of the vehicle were more difficult to make, but were 82±2 m for J. N. L. on January 23, and 95±4 m for E. R. C. and 92±3 m for J. N. L. on January 25. Thus, with reference to Fig. 1, it can be seen that a rear light smaller in diameter than 7.5 cm will be visible at a shorter distance than the vehicle carrying it and that within the range of the experiment the larger the rear light the further it will be seen.

The increase in visible range with rear light diameter can indeed be explained in terms of the angle it subtends at the eye (Fig. 2). The reduction in brightness contrast with distance can be calculated from Duntley's equations[4], for the brightness contrast presented by a black object near to the eye is -1 and the contrast perception threshold in good light is near 0.004. By comparing the visible range of the largest of the lights with that of the black surround and making appropriate provision for the angular subtense of both, it is possible to calculate the brightness contrast of the lights at their maximum visible ranges in terms of the angle they subtend at the eye. It is clear that these results compare well with those to be predicted from Blackwell's data.

In practical terms it would seem that most vehicle rear lights are too small and are indeed of very little use in thick fog in daylight. An increase in diameter up to about 20 cm would, however, result in an increase of 75–85% in the visible range that one vehicle can be seen from another.

I thank Mrs Erika Church and Miss Elaine Clarke for their help in preparing this work.

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BOOK REVIEWS

Piagetian Theory

Psychology and Epistemology: Towards a Theory of Knowledge. By Jean Piaget. Translated from the French by P. A. Wells. Pp. vii+107. (Allen Lane, Penguin Press: London, 1973.) £2.50.

IN his novel *Juan in America,* Eric Linklater remarks that the inhabitants of the American Middle West, though speaking English, think German thoughts. Piaget, on the contrary, though writing French, thinks English thoughts. This is nowhere more manifest than in this new collection of six essays from recent Piagetian writings.

The theme of the collection is the relationship between psychology and philosophy, though there are occasional remarks about the relationship between the former and other academic disciplines such as sociology and economics: but the main burden of argument turns on possible relationships between developmental psychology and epistemology and logic in particular.

It is Piaget's view that the concrete studies of psychologists, particularly developmental psychologists, can provide a kind of fundamental groundwork of hints for the conceptual analyses of operations and concepts which are the province of philosophers proper. The upshot is a kind of synthesis of what one might call the "Cambridge" and the "Oxford" ways. The Cambridge way looks to the formal analysis of concepts as the method of philosophy, while it has traditionally been the Oxford style to base all conceptual analyses in a set of very concrete exemplars. This perhaps is a way of representing the difference between the formalist and ordinary language schools of analysis in contemporary philosophy. In his inimitable way, Piaget succeeds in reaching a point of synthesis between these two main contemporary trends.

The synthesis is achieved by paying attention to the developmental history of a conceptual or logical operation. For example, Piaget shows in the case of number how some kind of synthesis between the Russellian conception of number as a set of sets, and the Peano conception of number as a simple ordering of objects according to a certain scheme, can be brought into some kind of relationship. Developmental psychology shows that a child first has to acquire the idea of a set from the notion of simple ordering. He will conceive of a set in the first instance only if it is capable of an organization of the Peano conception. Once the notion of set has thus been grasped, the child is capable of setting one set in one-one correspondence to another similarly derived. Finally, by an act of abstraction, the child comes to grasp that the order of the elements in the set is of no numerical consequence and that all that matters numerically is the one-one correspondence between like sets. In this way Piaget, in this and many other examples, sees the possibility of a synthesis between the formal and the concrete.

There are all sorts of other ideas developed in more or less detail in the book. Perhaps the most significant for the contemporary scene are his ideas about the possibility of inter-disciplinary studies. He envisages a very large number of combinations between sociology, linguistics, psychology, new forms of abstract engineering such as cybernetics and information theory, all related in one way or another to and through philosophical analysis. It is only at this point that one feels that Piaget's isolation within an essentially French-speaking world has led to him being somewhat surprisingly out of touch, though of course this may be due to the fact that these essays were written some years ago. He seems not to be aware that the project which he sketches of a thoroughgoing interaction between psychology, sociology and philosophy is already in full flood in what has come to be called "the ethogenic movement", derived from many sources, some of which, such as phenomenology, one would expect Piaget to be fully familiar with. One might say that he has identified the place where development can take place but has apparently not yet discovered that there is where the action is.

In one other and striking way these essays seem a little out of touch. They embody a curiously naive "laws in deductive structure" view of science, though Piaget is quick to repudiate an identification of his method with that of any form of logical positivism. In this way he shares the assumptions of many of his generation as to the proper analysis of science, while rejecting the logical positivism from which it ultimately derived. This is matched by an apparent lack of awareness of the rapid contemporary decline in confidence in both experimental psychology and formal methods of philosophical analysis, to both of which Piaget remains devoted. And yet it is clear that the spirit of the rapprochement between epistemology and developmental psychology that he advocates points strongly in the direction of the new and radical ideas as to what empirical psychology and philosophical analysis ought to be. In short, this book is as interesting and provocative as Piaget's more major works. One ought to remark finally on the excellence of the translation. As I said earlier in this review, Piaget's style of thought is, I think, more Anglo-Saxon than French, and perhaps this leads one to feel that in translation the book reads as if it were written directly into English.

ROM HARRÉ

Fourier Probabilists

Fourier Analysis in Probability Theory. By Tatsuo Kawata. Pp. xii+668. (Academic Press: New York and London, November 1972.) $32.

FOR many years now the theory of Fourier series and integrals has been a powerful tool for studies in the mathematical theory of probability. But as classical Fourier analysis has retired from the front of the mathematical stage, many probabilists find themselves ignorant of the possible refinements of the tool, and their mathematical armoury is correspondingly weaker. If this book succeeds in reminding practitioners of probability theory of the deep and powerful results of old-fashioned hard Fourier analysis, it will have performed a useful service.

Most of the book is a workmanlike account of the theory of the Fourier transform and its relatives in the classes L_1 and L_2. Although this treatment is written with applications to probability theory in mind, it includes many results which have not yet found such applications. Even the experienced reader may find himself in deeper water than he expects, and familiar theorems take fresh meaning from unfamiliar contexts and formulations.

The latter part is concerned with some areas of probability theory in which Fourier analysis plays a part. Pride of place naturally belongs to the continuity and compactness theory of characteristic functions, but the author devotes a good number of pages to topics related to the convergence of random series of different types. It might be argued that he could usefully have given a wider and more superficial survey. For example, the early parts of the book contain the prerequisites for the arithmetical theory of probability distributions (which has recently come into prominence again in the context of "delphic semigroups"). Again, there is no sign in this book of the very extensive work carried out in recent years, mainly in Eastern Europe, on the rate of convergence in central limit theorems and the theory of large deviations. Moreover, the Fourier theory is entirely classical, and there is for example no recognition of the importance of that elusive and challenging object, the convolution algebra of measures on the line.

These criticisms of course merely say that the author has not written a different book. The one he has chosen to write will prove most useful to any probabilist who wants to be able to use the tools of hard analysis, and who is not content to work on easy problems made to look significant by perverted abstraction. J. F. C. KINGMAN

Immunotechnology

Methodology of Immunochemical and Immunological Research. By J. B. G. Kwapinski. Pp. xx+820. (Wiley: New York and London, January 1973.) £16.60.

To the outsider the various branches of immunology could seem equivalent to the sects of a religion; the differences of observance can obscure the common theme. The pioneer immunologists were often medical microbiologists interested in the notion of immunity as a defence mechanism against invasion by microorganisms. Their efforts in the past have been remarkably successful in eradicating a number of previously common and serious diseases. In more recent years two further major trends in immunology have emerged. First, the nature of immunoglobulin antibody molecules has been delineated; and second, the origins of the immunologically responding cell populations have been revealed. The older formulation of immunology and the newer disciplines have each developed their own technologies. In this book by Professor Kwapinski, which is a more up to date version of his *Methods in Serological Research* (1965), it is elaboration of the older formulation with which he is principally concerned. An author should not necessarily be taken to task for the information given on the cover of his book but in this instance it must be pointed out that the title and the cover are misleading in that it is only a restricted view of immunology which is presented. For example there are two large chapters on antigens and their characterization which include nothing on histocompatibility antigens; the emphasis is almost entirely on bacterial and viral products. Thus the book is something of an anachronism but it may nevertheless be of value when the roots of immunological research are re-explored as they surely will be in the not too distant future. The illustrations are of poor standard and doubtful usefulness and the price is very high if the book is to serve as the "ideal text for students in medicine, dentistry, science and biological sciences"—to quote the cover. A. J. S. DAVIES

Behavioural Biology

Essentials of Physiological Psychology. By S. P. Grossman. Pp. vii+506. (Wiley: New York and London, 1973.) £6.65.

IN 1967 the author published *A Textbook of Physiological Psychology*, of 932 pages, and intended for graduates. Now five years later he has produced *Essentials of Physiological Psychology*, of 506 pages, and intended for an introductory course. The layout of this book is like that of the larger. It is divided into four main sections. The first (three chapters) is an introduction to neurophysiology and neuroanatomy. The second (five chapters) covers some of the senses, vision, audition and the chemical senses, together with chapters on the motor system and the reticular formation. The third section has seven chapters on motivation and emotion, dealing with hunger, thirst, sexual behaviour, emotional behaviour, reward and punishment, sleep and arousal, and finally with theories of motivation. The last section (five chapters) reviews work on the localization of capacities for learning, and the electrophysiology and biochemistry of learning. This book differs from the previous one in several respects other than its reduced length. It contains a chapter on research methods in the first section which briefly describes techniques of ablation and of electrical recording and stimulation. The other textbook had no chapters on any sensory system other than vision, and no separate chapter on sleep, arousal and attention. In the present book there is a brief mention of evidence on humans in the section on the hippocampus, whereas in the earlier book there was none. The last difference is the inclusion in this book of summaries and reviews at the end of chapters or parts of chapters, these being added, according to the preface, to make the book more suitable for an introductory course.

It is necessary to assess not only what is included but also what is left out from a book of *Essentials of Physiological Psychology*. Some of the omissions are serious. The chapter on research methods contains no section on behavioural techniques, and no section on experimental methods in neuroanatomy. Yet a student could hardly be said to understand a behavioural deficit if he does not know how the behaviour is assessed, or to understand the neuroanatomy of the brain if he does not know how it can be shown that area A is connected to area B. The chapter on neuroanatomy is in the worst tradition of human neuroanatomy textbooks, concerned mainly with labelling, and only with the human brain. The secrets of neuroanatomy for students of physiological psychology are not contained in the cranial nerves. This chapter is also very sparsely and poorly illustrated, there being, for example, no figures for the hypothalamus or thalamus. One last respect in which the book omits essentials lies in the very scant coverage of neuropsychological data on humans. No mention is made of language or specialization of the hemispheres, in spite of the fact that the neuroanatomy chapter is all on man.

It might have been expected that the author would not only have selected the essentials from the previous book but also have brought it up to date. The chapters covering areas in which he has done research are up to date, those on hunger, thirst, emotion and neuropharmacology containing 25% of references dated 1967 or later. But the chapters on audition, vision, the anatomy of learning, and the reticular formation, none of them subjects in which he has worked, contain only 3.6% of such references. The discussion in these chapters hardly contains the essentials. No mention is made of recent work on stereoscopic vision, the discussion of frontal association cortex is at least 10 years out of date, and there is only a single paragraph on posterior association cortex, omitting everything of importance.

A textbook for an introductory course should not only contain the essentials but also interpret studies in a way which will help the student to grasp their significance. This is not done, for example, in the discussions of the results of single unit recording in visual cortex, or in the passing mention of studies in which electrical stimulation of the same area of the hypothalamus in a rat may produce not only eating but also drinking or gnawing. Similarly possible differences between the effects of bilateral hippocampal lesions in man and other animals are mentioned, but no real attempt made to account for them. The reviews at the ends of chapters are of no help since they do little more than summarize. A student on an introductory course may find the chapters on motivation helpful, but for the rest he will not gain a grasp of the essentials of physiological psychology. There are other better and more interesting books which will help him to do that.

R. E. Passingham

Gases in Metals

Interaction of Metals and Gases. By J. D. Fast. Volume 2; Kinetics and Mechanisms. Pp. ix+318. (Macmillan: London and Basingstoke, January 1972.) £10.

In volume 1, published in 1965, Professor Fast covered the thermodynamics and phase relations of gases in metals. In volume 2 he deals with kinetics but excludes the topics of absorption, catalysis and oxidation on the grounds that these are adequately treated elsewhere. Volume 2 has only five chapters of which chapter 1, on solution of gases in metals, to some extent covers the material of volume 1 but with greater stress on the atomic interpretation of solution phenomena. The remaining chapters are on diffusion in interstitial alloys, internal friction in metals and the Snoek effect and interaction of interstitials with other lattice imperfections. The emphasis, therefore, is on the effect of foreign atoms on the structural and mechanical properties of metals.

By limiting his field, however, the author has again achieved a satisfying balance between theory and practice, as is appropriate to a professor who is also Chief Metallurgist to Philips Research Laboratories. Because the author intends the book for students of metallurgy as well as practising metallurgists, the theory of a particular phenomenon such as interstitial diffusion or relaxation is developed from elementary first principles, in both phenomenological and atomic terms and then applied to an abundance of experimental data on gas-metal systems. The many references to original work make this an invaluable source book for practising metallurgists. For students, who would benefit greatly from Professor Fast's clear expository style, I fear that the high price of the book for the restricted field covered may prove a deterrent. J. A. Pryde

Marine Geochemistry

The Changing Chemistry of the Oceans. Edited by D. Dyrssen and D. Jagner. (Proceedings of the 20th Nobel Symposium held at Göteborg, August 1971.) Pp. 365. (Wiley: New York and London, October 1972.) £9.

This volume contains most of the papers and the informative discussions from a Nobel Symposium at which the main theme was the effects of man's activities on oceanic processes. These activities are increasing detectably the rate of movement of certain elements in natural cycles and introducing significant inventories of substances hitherto absent or at negligible concentrations. Increased knowledge is needed of the background levels and perturbations against which such changes may occur, and the processes governing the behaviour of the chemical forms concerned. It is appropriate then that the proceedings were concerned as much with the changing perspectives of our understanding of marine geochemical and biogeochemical processes as with pollution which can only be interpreted in the light of such information.

The balance of subject matter reflects the emphasis placed on atmospheric interactions and pathways, in current marine chemical research. Wind-blown dust as a component in pelagic sediments and an influence on trace metal distributions is discussed by Chester. The importance of aeolian pathways for pollutants is brought out by Goldberg in a synthesis with many quantitative insights into human influences on the sedimentary cycle. The transfer of particles from ocean to atmosphere is examined by Chesselet, Morelli and Menard, who also discuss the atmospheric gaseous chlorine, probably as hydrogen chloride, of marine origin. Hahn shows that parts of the Atlantic Ocean act as a source for atmospheric nitrous oxide also. The more fully explored carbon dioxide cycle is discussed here by Machta, with emphasis on the human perturbations of the inventory and seasonal atmospheric variations, and by Pytkowicz, who considers the long-term geochemical aspects. An important and rather neglected aspect of the air-sea boundary layer, namely the influence of surface films, is examined by Garrett.

Some information on changes in the chemistry of sea water on both recent and geological time-scales is recorded in the sedimentary deposits. Lal and Krishnaswamy describe investigations of ferromanganese concretions which, because of their dominantly authigenic character and slow kinetics of formation, may constitute especially valuable sedimentary records. Sedimentary pyrite formation and its quantitative importance as a removal route for oceanic sulphate is discussed by Berner. Several papers describe the occurrence of specific pollutants and the behaviour of elements which can cause pollution problems in coastal waters. Some useful contributions give relevant information on physical, meteorological and biological processes. The importance of modelling as an aid to understanding highly complex environmental systems emerges as another pervasive theme, to which Odum contributes an outstanding and thought-provoking study of energy circuit modelling.

The papers were published only fourteen months after the symposium. Misprints and eccentricities of presentation, numerous in some parts, might have been removed at the cost of a longer gestation. Although the uninitiated may ponder on page 326 over the nature of the testes which are said to fall rapidly to the sea bed and dissolve, these errors do not seriously detract from the value of the publication. It deserves a wide readership.

J. D. Burton

Irradiated Polymers

The Radiation Chemistry of Macromolecules. Edited by Malcolm Dole. Volume 1. Pp. xiv+369. (Academic Press: New York and London, November 1972.) $23.

Polymers, like materials of other kinds, are prone to suffer damage when exposed to high energy radiations but it often is the case that the effects can be turned to good use. For example, crosslinking can be induced by suitable irradiation of a moulded plastic article in order to stabilize its shape and enhance its mechanical strength, a reaction distinguished from other post-treatments of comparable types in taking place throughout the specimen rather than in limited depth at the surface.

Great interest attaches to the radiation chemistry of polymers at the academic level as well as the technological; previous accounts of the subject are now a decade out of date and the volume under review is the first half of a two-part treatment which is obviously timely, if not overdue. To some extent this volume is preparatory in that it deals for the most part with the theoretical aspects of the subject, including the concepts of energy transfer in polymers, the nature of free radicals, molecular mobility and electrical conductivity.

There is also a chapter describing experimental techniques and the book ends with two discussions of radiation effects in relation to polyethylene. These latter contributions seem rather out of place after the very general foregoing matter, but they are really a prelude to the second volume which will deal with a whole range of polymers, including crystalline, fibrous and biological materials.

The two final chapters really whet one's appetite for volume 2: they indicate the remarkable way in which the behaviour of high polymers can depend critically on minute details of structure and, conversely, on the way in which minimal changes in structure can produce drastic modification of properties.

While it seems unlikely that the earlier established works by Chapiro and Charlesby will become redundant, it is obvious that this book and its successor will be essential for any comprehensive library in a polymer science environment.

A. D. Jenkins

Essential Photosynthesis

Photosynthesis. By D. O. Hall and K. K. Rao. Pp. 68. (Edward Arnold: London, November 1972.) £1.50 cloth; 75p paper.

In this text (*Studies in Biology*, No. 37) intended for sixth-formers, their teachers and first-year undergraduates, Hall and Rao have set out a description of photosynthesis that is admirably clear and terse. After an introduction and history, they concentrate on sub-cellular aspects and explain light absorption (chapter 4) and electron transport (chapter 5) in chloroplast membranes (chapter 3) and the metabolic assimilation of CO_2 in their immediate environment (chapter 6). A comparative outline of bacterial photosynthesis follows (chapter 7).

This account, necessarily complex, is illustrated by a well-chosen series of electron micrographs and by clear diagrams explaining some crucial historical experiments, and others by which the student could corroborate the text. Recipes are given for chloroplast isolation and in chapter 9 some 15 practical experiments are listed (by reference).

The set length has restricted the discussion to the area which the authors can claim to be reasonably agreed. In chapter 8, however, there is a list of some of the regions where current research is most active. The suppression in the interests of brevity of details of, for example, the nature of redox carriers, or metabolic intermediates, also has some compensation in that each pathway can more readily be perceived as a whole by the student.

This approach must be successful provided that the text is used in situations where basic teaching can be relied on to establish the concepts of metabolic maps, electron transport, ATP and so on. In turn, the details that are provided will serve as an index for the student to enter the major literature and to tackle the many controversies yet to be resolved.

R. P. F. Gregory

Frege on Geometry

On the Foundations of Geometry and Formal Theories of Arithmetic. By Gottlob Frege. Translated and with an introduction by Eike-Henner W. Kluge. Pp xlii+163. (Yale University: New Haven and London, November 1971.) $10; £4.75 cloth.

Frege's articles on the foundations of geometry which are translated in this volume had their origin in a correspondence with David Hilbert about Hilbert's famous Festschrift. In the Festschrift, Hilbert constructed an axiomatic system for geometry and sought to prove the independence of the parallel axiom. Frege does not concern himself with the technical details of the system but with the fundamental questions whether the concepts of geometry can be defined intrinsically by means of axioms. In his reply to Frege's first letter Hilbert states his position in the following words: "If the arbitrarily posited axioms together with all their consequences do not contradict one another, then they are true and the things defined by these axioms exist." Frege contests this view in its entirety, arguing that there are no means of proving consistency other than by exhibiting an object with the properties demanded by the axioms, and if one has such an object what need is there to prove its existence by the roundabout method of consistency? Hilbert does not continue the correspondence, but the attack on Frege is taken up by Korselt who reiterates the standpoint of "modern mathematics". Frege's reply to Korselt is highly abusive: "I have been at pains," Frege says, "to draw sharp boundaries: Mr Korselt, it seems, diligently blurs them once again. How is this to be explained? Perhaps by a drive for self preservation on the part of Hilbert's doctrine for which an obscurity of the issue may well be a condition of survival." Again later in the same article he criticizes Korselt's changing conceptions of axioms, finding at least four different uses of the term. Frege rejects the axiomatic method for its failure to give references (denotations) to the words "point", "line", "plane" and so on, demanding of a definition (of a point for instance) that we will be able to judge by it of any object whether or not it be that which is defined. The kernel of Frege's opposition to formalism is that the propositions of mathematics are not mere sequences of words or groups of signs, but are thoughts, something which can be true or false.

In addition to the articles on the foundations of geometry the translator has included Frege's controversy with his Jena colleague J. Thomas about formal theories of arithmetic and contributes a thirty page introduction which sets the scene and fills in the background to the controversies.

The subtlety of Frege's mind and his tremendous gifts for logical analysis and disputation are boldly displayed in the essays in this collection, but the judgment of history has gone against Frege and the axiomatic method in mathematics has prevailed against all the fury of his attack.

R. L. Goodstein

Quantum Theory

The Quantum Theory of Atoms, Molecules and Photons. By John Avery. Pp. xv+378. (McGraw-Hill: Maidenhead, September 1972.) £8 cloth; £5.50 paper.

The quantum theory of atoms, molecules and photons forms a central part of undergraduate and graduate courses in physics and chemistry. There are a number of very good textbooks covering this field, so it is reasonable to ask if a new one is needed. No doubt we could do without this book, but it will surely find some friends, particularly among physical and theoretical chemists.

The author is a graduate of the Massachusetts Institute of Technology and of the Universities of Chicago and London, and is a lecturer in the Chemistry Department at Imperial College, London. The book has been written for chemistry students who like mathematics. It uses advanced mathematical techniques when these are needed but it does not assume too much prior knowledge. The style is lucid and pleasing, although the first chapter, on the development of mechanics, is rather long.

There are nine chapters covering topics such as Hilbert space, angular momentum, the chemical bond, translational symmetry, vibration and rotation, and "ions and ligands". There are seven appendices of which the largest is a 23-page account of group theory. A few examples are given and some have hints for their solution. The book provides a modern approach to many topics of interest to chemists and physicists—phonon and photon creation and annihilation, the scattering of radiation, optical activity, and semi-empirical computations of molecular structure and properties. Some of these and other topics are only lightly touched upon, but the overall coverage will appeal to theoretically inclined research students in chemistry and to a very few undergraduates. The charm of the book is enhanced by amusing historical and biographical anecdotes. Very few mistakes were noticed, although Russell, who is often linked to Saunders, is misspelled throughout.

A. D. Buckingham

CORRESPONDENCE

Club of Rome

SIR,—The editorial in *Nature* of March 16 (**242**, 147 ; 1973) gives a brief review of the voluminous report of the Science Policy Research Unit of the University of Sussex on the *Limits of Growth* (*Futures*, March 6), and calls it, hopefully, "Almost the Last Word on the Club of Rome". This is liable to give a false impression of the Club of Rome, of the *Limits* and to some extent even of the Sussex Report.

In my opinion the Sussex Report is not so much an axe as, rather, so far the best constructive critique of the *Limits*. It is fair, courteous and thorough. Marie Jahoda in her "Postscript on Social Change" writes, "We know from our experience in studying Forrester's and Meadows's work that it took a group of highly trained experts in a variety of fields many months of hard effort to grasp fully the technique, the assumptions and the shortcomings of world dynamics". Almost every page of the Report bears this out. It is a very welcome change after the wholesale rejection of *World Dynamics* and the *Limits* by many economists, whom one must strongly suspect of not having read the books, but just leafed through them with disgust. The Sussex Group have read them, digested them and much of what they write, though not all of it, can be considered as a fair commentary, which will give the reader a deeper understanding of these works, while showing up some of their weaknesses.

The least satisfactory are the computer runs of the Sussex Group. They cast doubt on the reliability of the world models by running them backward, with the result that they "retrodict" a catastrophe in the past. I need not deal with this, as it has been answered by Meadows in the same number of *Futures*. An error of 1/1,000 in some parameters is liable to make the model run off its track in the past, while an error of the same order would be hardly noticeable in a forecast. The Forrester–Meadows models share this property with many other complicated physical systems. Nor need we be impressed with the difference which they find in forecasts by substituting expanding in place of shrinking resources. This is not a "small change" in the assumptions.

On the other hand, in view of what we have experienced from economists, it is an agreeable surprise to find that chapter 6, *The Capital and Industrial Output System* by Christopher Freeman with the economists Julien and Cooper, is particularly constructive. They make it clear that it is hopeless to treat an aggregated system, in which Africans and Americans are lumped together, dynamically with a single set of operators. This is macroeconomics taken too far. One cannot expect from such a forcibly homogenized model anything but coarse approximations, and nothing more has ever been claimed by its authors.

This type of critique, of which there are many more examples in the long report, is welcome to the Club of Rome as a dialectical aid towards the next step. It does not find us at all unprepared. We have never considered the world models as oracles of unescapable fate. Perhaps the words "Predicament of Mankind" were misleading. A predicament is not a trap from which one can never escape ; it was meant to be a warning and a challenge. A warning against "ostrichism" and a challenge to take up the fight with the dangers which have been revealed. As far as I can see, the Sussex Report nowhere states that the dangers are imaginary, though it often suggests that they might be exaggerated.

Are they exaggerated? I think that if the Club of Rome has anything with which to reproach itself, it is rather that it has not warned in time that while the global danger may be a hundred years ahead, its first serious wave is right upon us. We are far from living in a unified world in which the resources are equitably or at least reasonably shared. While the Club of Rome warned of an overshoot in the consumption of non-renewable resources some time in the next century, the highly industrialized nations and in particular the United States have long overshot the limit at which they could live safely within their own means. A grave fuel and energy crisis is now striking at the industrialized countries, long before the resources of the world are exhausted—because we have not foreseen it in time.

The fuel and energy crisis which is starting now is likely to last for two decades, and it is scant satisfaction for us of the Club of Rome that from now on we are likely to meet much less complacency, much less of the "I am all right, Jack!" attitude. When the Sussex Group states that it considers the political difficulties as more urgent and important than the physical limits, it is in perfect agreement with the Club of Rome. From the start its founders have considered international cooperation as their most important target, and they were successful in establishing a worldwide network of intellects, not entirely without political influence.

There are many minor deficiencies in the Forrester–Meadows models which could be amended, but it is clear that no aggregated world model can be satisfactory. The Club of Rome has long understood this, and now, among other projects, it is sponsoring a *regionalized* computer model of the world. This ambitious project, headed by Professors Mesarovic and Pestel, divides the world into 10 regions, taking account of their self-interest and also of their political philosophy. If successful, this project may give answers to many questions which the *Limits* have left open. Only one thing is certain ; the dangers, the instabilities, will be found very much nearer to us than the global physical limits.

As regards the ultimate, physical limits, we believe that these can be pushed further away, but we are not satisfied with pious hopes. The Club of Rome is going ahead with plans for mobilizing creative technology in all countries. We are not "doomwatchers" but doomfighters.

Yours faithfully,

DENNIS GABOR

Imperial College,
London

Princeton in Trouble

SIR,—Many of us at the Institute for Advanced Study were sad to read your leader about Princeton (*Nature*, **242**, 217 ; 1973). Not only is your statement of the facts extremely misleading, but also your underlying philosophy is difficult to understand. Your writer seems to believe that an academic institution, by its very nature, should be a dictatorship, and that in case of a conflict between faculty and director the faculty must go.

The background of the present dispute can be described briefly as follows. It had been unanimously agreed that the faculty as a whole should supervise the formation of the fledgling "Program in Social Sciences" by examining the credentials of candidates for the first three professorships. Precisely because these first appointments would determine the future direction of the program, it had been agreed that such faculty supervision was essential ; and in fact the program got off to an excellent start with the unanimous faculty approval of Clifford Geertz as the first professor.

Going further back, the following promise was made in 1947 by Frank Aydelotte, then director of the Institute: "I have given an undertaking to the faculty that I would never recommend to the trustees an appointment of which the faculty did not approve". This principle has been a firm tradition ever since, until the present altercation. It is our hope that negotiations now in progress between faculty and trustees will lead to a reaffirmation of Aydelotte's undertaking.

For readers who may have been misled by your headline, let me emphasize that the Institute has no connexion with Princeton University, which has a long record of excellent administration.

Your faithfully,
JOHN MILNOR

The Institute for Advanced Study,
Princeton, New Jersey 08540

Careers

SIR,—The worsening market situation for scientists and technologists described in your supplement, "Careers for Scientists", and the threat of further reductions in scientific establishments, especially in the largest companies, leads to one irresistible conclusion: the need for scientists and technologists to join this union set up by the Council of Science and Technology Institutes just over a year ago. It is one reason why membership is already 4,000 and climbing steadily.

Of course, as several of your contributors pointed out, the picture is not all black. More and more science graduates are entering managerial employment, which we believe to be essential if the science-based industries are to develop their full potential, or scientific work at a supporting level. Viewed, therefore, as a whole, we would agree with Mr Davies that we are not producing too many highly educated people and can do with more, provided that planning is sensible and that people are told what to expect. This, too, emphasizes how vital it is that scientists and technologists moving into unfamiliar fields make sure that they can avail themselves of the assistance which APST can provide.

Yours faithfully,
M. I. GILLIBRAND

Association of Professional Scientists and Technologists,
Hinchley House,
14 Harley Street,
London W1N 2BE

Citation and Distinction

SIR,—Garfield[1] continues to make superficially plausible claims for the Science Citation Index, which his company publishes. Obviously Nobel Prize awards and citation frequency are partly caused by the same factors. It may be coincidental that Nobel Prizes are not given in the evolutionary half of biology (with adjacent fields such as stratigraphic geology), where the literature is extraordinarily diverse and is poorly represented in the Index.

In Garfield's lists[2] of most-cited and highest-impact journals, eighty-one and seventy-one respectively of the first 100 are devoted exclusively to chemistry, physics, biochemistry, physiology, and medicine. This partly reflects the same bias in the source, but also the fact that there are, for example, more organic chemists than evolutionary biologists.

In a forthcoming work where I propose a new scientific law with considerable applicability, I make few references. But the result depends directly on, and would have been impossible without, a large proportion of all the work in evolutionary biology that has been done in the past. A simple-minded cost-effectiveness approach like Garfield's would have prevented this work from being done.

Any action taken on the basis of such biased results as Garfield's clearly discriminates against the areas under-represented. These areas are as a whole probably more dependent on extensive availability of literature than is any other area of science.

Yours faithfully,
LEIGH VAN VALEN

Committee on Evolutionary Biology,
The University of Chicago,
5734 Ellis Avenue, Chicago,
Illinois 60637

[1] Garfield, E., *Nature*, **242**, 485 (1973).
[2] Garfield, E., *Science*, **178**, 471 (1972).

Human Papova Viruses

SIR,—We would like to correct some of the facts in your News and Views article "Human Papova Viruses" (*Nature*, **241**, 308; 1973).

A human papova virus of the polyoma virus subgroup "first came to light" when Zu Rhein and Chou observed by electron microscopy virus particles in brain cells of patients with progressive multifocal leucoencephalopathy (PML) (*Science*, **148**, 1477; 1965). Many attempts have been made to grow this virus *in vitro* without success.

Simultaneously with our report of the isolation of BK polyoma virus from the urine of a renal transplant patient (Gardner *et al.*, *Lancet*, i, 1253; 1971), Padgett and her colleagues described the successful culture of JC polyoma virus directly from the brain of a patient with PML (Padgett *et al.*, *Lancet*, i, 1257; 1971). It was unfortunate that no reference to this important work was made in your article. Later, Weiner and his coworkers reported the isolation of two further strains of polyoma virus from the brains of two patients with PML (*New Engl. J. Med.*, **286**, 385; 1972). Both of Weiner's isolates appear to be antigenically identical and biologically similar to the simian polyoma virus (SV40) whereas JC, the other virus isolated from PML, appears antigenically unrelated to SV40 by immunofluorescence.

Our BK polyoma virus has only a very minor antigenic cross-reaction with SV40 and shows many differences from this virus biologically, including the property to agglutinate human O and guinea-pig erythrocytes to high titres. It seems therefore that we are indeed dealing with a group of different human polyoma viruses. In the discussion of human papova viruses it must not be forgotten that human common wart virus is also a member of the papova virus group although it belongs to the papilloma virus subgroup.

Yours faithfully,
SYLVIA D. GARDNER
ANNE M. FIELD

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Announcements

Miscellaneous

Elections to the US National Academy of Sciences:

Edward Hamblin Ahrens, jun., **Rockefeller University**; Robert Wayne Allard, **University of California, Davis**; Andrew Alm Benson, **Scripps Institution of Oceanography**; Howard Alan Bern, **University of California, Berkeley**; James Daniel Bjorken, **Stanford University**; Harold Charles Bold, **University of Texas**; John Tyler Bonner, **Princeton University**; Frederick Herbert Bormam, **Yale University**; Gordon Howard Bower, **Stanford University**; Felix Earl Browder, **University of Chicago**; Donald David Brown, **Carnegie Institution of Washington**; Arthur Earl Bryson, **Stanford University**; Bernard Budiansky, **Harvard University**; John Werner Cahn, **Massachusetts Institute of Technology**; Robert Merritt Chanock, **George Washington University**; Albert McCavour Clogston, **Sandia Corporation**; Ansley Johnson Coale, **Office of Population Research, Princeton, New Jersey**; George C. Cotzias, **Brookhaven National Laboratory**; Philip Ernest Converse, **University of Michigan**; Ellis Brevier Cowling, **North Carolina State University**; James Edwin Darnell, jun., **Columbia University**; Albert Dorfman, **University of Chicago, School of Medicine**; Otis Dudley Duncan, **University of Michigan**; Isidore Samuel Edleman, **University of California Medical Center, San Francisco**; Walles Thomas Edmondson, **University of Washington**; Edmond

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Time for a Cod Peace

THE cod war took a turn for the worse last week with a British trawler being holed by fire from an Icelandic gun boat. Relations between Britain and Iceland are now strained to their utmost and it is clear that the sooner negotiations are resumed between the two countries the better it will be for both governments and fishermen. In the circumstances it is unfair for Iceland to insist that the British navy departs before talks restart.

The issues are clear enough at this stage and Iceland's demand to control the fishing within fifty miles of its own coast is mostly a means to ensure, as best it can, the future economic growth of the country. It is agreed that the cod in Icelandic waters is fully exploited and for Iceland itself to obtain greater benefit from the seas nearest to its shores, other countries must restrict their catches in these waters.

The British fishermen see this as yet another attempt to deprive them of good fishing grounds. About a third of all the cod brought into British ports in 1971 was obtained from the waters around Iceland and in contrast to other areas in the North Atlantic where cod is traditionally found—such as the North East Arctic region—the waters are not at present over fished. There is little hope of obtaining cod further than fifty miles from Iceland for most of the cod is found on the hundred fathom deep continental shelf within the fifty mile limit.

Cod fishing in the north Atlantic is controlled by two commissions, the International Commission for North West Atlantic Fisheries and the North East Atlantic Fisheries Commission. Britain and Iceland are members of both bodies. Part of the present troubles stems from the decision of the North West Commission to limit the catch in the waters under its control, not by the traditional method of altering the size of the fishing net mesh but by imposing a limit on the catch. These controls only affect the fishing off the west coast of North America at present. These controls were introduced at the beginning of 1973 but the efforts of the North East Commission to introduce similar restrictions have been thwarted by no other country than Iceland which only two weeks ago refused to accept a similar agreement drawn up by the North East Atlantic Fisheries Commission.

These developments, coupled with the introduction in recent years of factory ships—that is trawlers with refrigerated storage—have convinced the Icelandic Government that the waters near its own shores would be the first to be put under pressure if tight limits were imposed in the North East Atlantic, in particular the seas to the north of Norway and to the south of Spitsbergen and the Barents Sea, which have been over fished.

But is the cod in danger of being fished to extinction in the North Atlantic? Clearly the cod is not threatened to the same extent as the whale for example. Whales reproduce once or twice every one or two years but a large cod could lay several million eggs during its lifetime. There is a need for only two of these eggs to reach maturity to ensure that the stock is replaced. The size of the mesh now used by cod fishermen—130 millimetres—ensures that no cod is caught until it is at least two years old and the rate of fishing is such that it is estimated that about fifteen per cent of two year old cod will survive for seven years, the age of full maturity. But, it must be said, there is a fine line between fishing enough to ensure that the stock is replaced and fishing to ensure that the stock will not become depleted. At present the North West Commission is advised by scientists as to the amount of fishing that can be done and the commission then divides this up among the representative countries according to the amounts of cod which they landed during previous years. What is at once clear is that the anomalous situation with only one of the fishing commissions setting limits on catches will impose a great deal of pressure on the fishing grounds under the aegis of the other commission and, whatever happens about Iceland's claims, the two commissions should, at least, present a common policy on catches. In particular setting a limit on the cod that can be caught in the Arctic regions north of Norway will ensure that these grounds will recover properly and will not be over fished again during the recovery period.

But how will this help settle the dispute over fishing within fifty miles of Icelands shores? The Icelandic waters are, by mutual agreement, fished as much as is wise already. Setting quotas on the fish taken from these waters will not necessarily alter the total catch. More evidence is needed soon to decide whether Icelandic waters are being overfished or not.

It must be faced that Iceland badly wants to improve its economy. Fishing is the country's chief industry and an improvement in this sector can only be obtained by an increased catch which, without seriously damaging the stock, can only be achieved at the expense of another country's catch. Can a way now be found over the negotiating table to aid Iceland without seriously damaging the livelihood of the British trawlermen?

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100 Years Ago

NATURE

PROFESSOR WYMAN has concluded, as the result of explorations among the shell mounds of Florida, U.S., during the past winter, that the aborigines by whom they were constructed must have been decided cannibals, as in eight different instances he has found considerable quantities of human bones in the shell heaps, the bones themselves being broken up and split, just as in the case of the bones of other animals. This, he is satisfied, was not the result of burial, but was done for the purpose of obtaining the marrow, probably after the flesh had been devoured.

From Nature, **8**, 112, *June* **5**, 1873

OLD WORLD

"European Science Foundation Inevitable" Sir Brian says

A DEAL of European banner waving was apparent in Berlin last week when Sir Brian Flowers, Chairman of the Science Research Council, delivered the Queen's lecture and announced that "I now believe that the creation of a European Science Foundation is not only desirable but inevitable and that it will happen quite soon".

Sir Brian, who declared that he was out to be "frankly political" although "only in the context of European Science", outlined how he thought the new foundation should work and what it should do.

The foundation was originally proposed in its present form by Commissioner Spinelli of the European Commission in June last year as a body "that would not be designed as a substitute for existing centres and associations, but to support their actions and contribute to their effectiveness by way of coordination". Its particular tasks would be to ensure the mobility of research workers, to organize international meetings, support European activities and projects, finance laboratories that have the potential of being European centres of excellence and examine national plans for large research facilities with a view to their being funded on a cooperative basis in the future.

Sir Brian clearly approved of these aims, but he went on to underline certain points he feels to be important. Membership of the foundation must not be limited to members of the community, he said. "Scientific cooperation cannot be limited by national borders" and limiting cooperation could submit fundamental science to political and economic goals which are often too immediate to be relevant to scientific and cultural pursuits.

The foundation must also be autonomous. Finance must come both from the community and from the national organizations that adhere to it, and its scientific judgements and policies must remain in the hands of the scientists themselves. Equally, Sir Brian said, the initiatives must come from outside the foundation rather than from its bureaucracy. As the Max Planck Society has put it, fundamental research "should rather be supported than organized, and rather promoted than programmed".

To help ensure this the commission's share in the foundation, important though it is, should not be too great. More than half the finance should come from national organizations such as the research councils.

The foundation's budget should therefore be—at least at the beginning—a small one. "Major expenditures" Sir Brian said, "should come, as they do now, from coordinated national funding". This will avoid the twin dangers of financing projects purely for political reasons "merely in order to appear European", and of supporting big science (space research, astronomy and particle physics) which can only be funded internationally, to the detriment of little science such as botany, which can be funded locally.

Sir Brian was equally adamant that cooperation should take place on the small as well as the grand scale. University departments across Europe should cooperate with each other, and indeed "the research councils are already trying to encourage such developments".

The other key role that Sir Brian envisages for the foundation is that of supporting European learned and professional societies. Citing the example of the European Physical Society, which is currently short of cash, Sir Brian said that the relatively trivial sums required to finance such bodies are proving hard

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BACTERIOLOGY

What's in a Name?

THE nomenclature of bacteria is likely soon to be revised. After five years work a committee of bacteriologists has produced a series of proposed revisions to the International Code of Nomenclature of Bacteria[1] that could result in ninety per cent of the published names for bacterial taxa disappearing by 1980 as a new list is drawn up.

This somewhat extreme solution, however, is really only a measure of the problems that face bacteriologists. The International Code of Nomenclature of Bacteria lays down that taxa must always be known by their earliest name, with 1753 being taken as the earliest acceptable date. This has led to lengthy searches in dusty literature by systematists who have to check that they have found the earliest name for a group of organisms. But, not surprisingly, the first descriptions of microorganisms are often so poor by modern standards that their identity now is a matter of opinion rather than of fact, and a number of names are being conserved by international agreement when all they are doing is creating work for the people the code is meant to help.

The solution that has been proposed by a five man committee headed by Dr S. P. Lapage of the National Collection of Typecultures is that between now and 1980 a list should be prepared of all names for bacterial taxa that have any claim to be worth keeping. The remainder—perhaps ninety per cent of the old names—will be forgotten. Thus if no one cares enough about an old name for a species that in fact cannot now be clearly recognized, it is better to forget it and allow it to be rediscovered and renamed if need be. By 1980 only the names on the new list will be used.

In an attempt to prevent a similarly chaotic situation building up in the future, Dr Lapage and his committee have also recommended that any new names must be published in the *International Journal of Systematic Bacteriology* if they are to be valid.

Since, from 1980, the new official list will be published in that journal, this means that all these and all future names will be readily available in one publication. Clearly the committee cannot legislate that all descriptions of new taxa must appear in one journal, so a distinction that already exists in the code between "effective publication" and "valid publication" has been exploited. "Effective publication" is publication of a description anywhere in the scientific literature, but for it to be "valid" the name must also be published in the *International Journal of Systematic Bacteriology* with a reference to the description if that has been published elsewhere.

At first sight the solution seems neat and elegant. In spite of the size of the problem, bacteriologists are fortunate in having a relatively small number of species to manage. Botanists and zoologists have similar problems, but the range of species involved may make a similar solution impracticable.

Comments on the new proposals will be considered during the summer and the revised code will go before the International Congress of Bacteriology for approval this autumn. Assuming the congress approves, bacteriologists may, by 1980, be able to spend less time combing dusty shelves.

[1] *International Journal of Systematic Bacteriology*, **23**, 83–108.

to find from national sources and should be made available through the foundations.

The foundation should also be given the task of fostering European professional institutions as well as learned societies. Such bodies should help establish confidence in the professions, and would, Sir Brian implied, go a long way to solving the problem of what qualifications can be recognized throughout Europe—a problem with which the Commission has struggled long, hard and fruitlessly.

But before outlining his ideas on how a European Science Foundation should operate, Sir Brian pointed the lessons that can be learnt from Europe's attempts at scientific collaboration over the past twenty years.

To start with, Sir Brian said, the science to be undertaken must be good science. The initiative for the new organization must come from scientists, not politicians, and the objectives must be clearly defined. Particularly in technological fields the overall policies within which the aims of the organization are set must be clearly understood—"there should be no projects without policy as was the case when Euratom was created".

Further, the cost or content of programmes must justify the cooperation. "It is not enough that we have problems in common; we have to show that we are better able to solve these problems jointly rather than separately".

Once a European organization is set up, however, each country "should give its support of that organization priority over any residual national effort in this field . . . national programmes should wherever possible exist, but they should be supportive or complementary, and clearly seen to be so".

Sir Brian also underlined the all too familiar dangers of a rigid policy of "juste retour", whereby each country expects to recover, through contracts placed within its national industries, the money it has put into cooperative organizations. The policy is "nothing but a wasteful irritation", Sir Brian said. "With the growth of European companies it is in any case likely to become an irrelevance of no economic significance and of dubious political value".

TELEVISION

Angels Not Apes

Dr Jacob Bronowski's television programmes on the BBC are certain to be a sensation. The title, *The Ascent of Man*, shows where he stands. Not for Bronowski a downbeat Darwinian word like descent. Instead, he is ebulliently, unfashionably but not outrageously anthropocentric. The first of the programmes, three weeks ago, put the point directly. Australopithecus was above all intelligent, and intelligence made it possible for even australopithecenes to invent cultural adaptations to different kinds of habitat. The result is that their offspring genera have inhabited the world and that the Lapplanders whom the BBC has taken the trouble to film exquisitely are intrinsically smarter than the reindeer on which they live, partly as nomads and partly as neolithic agriculturalists. Dr Bronowski's illustrated hymn to the cleverness of people is in part at least intended as challenge to the contemporary mood of those who hold that all species are intrinsically equal, like numbers in a Bingo game. It is an eloquent challenge. Altogether there will be thirteen programmes. The film is everywhere superb. So, in his idiosyncratic way, is Bruno, as they call him. Alone among television performers, he has made the pause for words a source of tension and a vehicle for excitement. And, as always, he takes the grand view. The BBC has labelled this series of films *A Personal View* and that is understandable, not necessarily a piece of cowardice. For many of Bronowski's messages are open to argument. What matters is the grand conception and there is no doubt that the BBC now thinks, with some reason, that it has on its hands a fitting counterpart to the series of films by the then Sir Kenneth Clark labelled *Civilization* which omitted to mention technology other than that invented by Leonardo da Vinci.

In principle, the BBC is right and the new series is a triumph for it as well as for Bronowski. Will the quibbles that can be raised become the scenario for another series? Precisely how did the Australopithecenes escape from East Africa (a programme of research, not a question for the BBC)? Might it not have been sharper, in the third programme, concerned to show how building has been a constant outlet for inventiveness, to have played church music for the Watts towers and George Gershwin for cathedrals (a question for the BBC, not for Bronowski)? But even if it is right, as the eleventh programme will in due course argue, to infer from the uncertainty principle and the doctrine that nothing is absolutely knowable that people should be tolerant of other people's ideas and even other people, is there not some logical gap to be bridged?

Where does Bronowski stand on Jensen–Eysenck? And may there not be more to be said about tolerance (and the need of it) than can be done knee-deep in a mud of funerary ashes in an Auschwitz pond (which is a question for Bronowski)? That said, the programmes are enough of a sensation for *Private Eye* already to have begun to mock them and they will make a mark.

DRUGS

Safety Suggestions

Recommendations for international drug safety legislation have been formulated as a result of a conference held in Italy recently. The special commission on internal pollution, a collection of scientists representing most of the countries in the European Community, was organized by Sir Rudolph Peters, University of Cambridge, who took the chair. Mr Jean Rey, the Belgian Minister of State and recently president of the EEC, proposed that Brussels should be approached unofficially to consider establishing a voluntary body to harmonize drug safety evaluation within the community.

The recommendations made include the development of clinical pharmacology, which in many EEC countries is not regarded as a speciality, and a greater degree of integration between animal and human studies. Compounds should be considered for study in people as soon as experimental work indicates potential therapeutic value and a lack of significant side effects. Human studies should then dictate subsequent animal experimentation.

Value Added Science

Financial musical chairs between the Treasury, the research councils and their customer departments have marked the introduction of the financial changes promised in last July's White Paper on government research and development which came into effect on April 1—the same day as Mr Anthony Barber's Value Added Tax.

The Department of Education and Science's budget was reduced by the Treasury by the amounts that the three research councils involved in the Rothschild cuts were due to lose to their customer departments. In the case of the Agricultural Research Council this amounted to £6.5 million which the Treasury gave instead to the Ministry of Agriculture, Fisheries and Foods. However, as the ministry and the ARC have not yet agreed which projects the ministry is to count as part of its contract research with the ARC, MAFF simply transferred the £6.5 million straight across to the council. Only by this time the £6.5 million had beome £7.2 million as the council has to charge the ministry VAT as contract research is a service. The extra £0.65 million will shortly be transferred back to the Treasury which provided the money to MAFF in the first place. It keeps the civil servants busy.

NEW WORLD

Cries of Distress from the Medical Schools

by our Washington Correspondent

FOR the past few years medical schools in the United States have been caught in a financial squeeze and there has been persistent talk of budgetary crises. But the talk turned to cries of anguish in January this year when President Nixon's budget for 1974 was published—for the first time, the schools are faced with an absolute cut in federal funds, and with the abolition of some cherished programmes. Exactly what effect the proposed cuts will have on the medical schools has, however, been a matter of some debate, and so the Association of American Medical Colleges (AAMC) has conducted a survey of its members to find out. The results, published last week, confirm the worst.

According to replies from 78 of the 113 medical schools in the United States, the proposed funding cuts will result in a reduction in staff of at least 1,400—one out of twelve faculty members—possible reduction in enrolments, curtailment of some programmes designed to assist minority and disadvantaged students and a variety of other cost-cutting measures. Small wonder that Dr John A. D. Cooper, president of the AAMC, last week called the proposed 1974 budget "a serious blow to medical education, biomedical research and health care in this country".

Although the medical schools receive funds from a variety of sources, they have become increasingly dependent on the federal government for their money: in 1972, for example, the government provided 44 per cent of the total income of the medical schools. But dependence on federal financing has made the schools particularly susceptible to vagaries in the budget, clashes between Congress and the White House and the whims of officials in the Office of Management and Budget.

In the early 1960s, their finances were relatively healthy, largely because of the rapid increase in the budget of the National Institutes of Health and the consequent flow of research money into the universities and their medical facilities. But the schools' troubles began with the levelling off in research funds of the late 1960s and early 1970s, went from bad to worse when Congress and the White House failed to reach agreement on the appropriations bill for the Department of Health, Education and Welfare last year (see *Nature*, **242**, 81, 1973), and will deteriorate even further if the Administration's plans for next year are carried out. The schools' financial ills are also exacerbated by the fact that they have nearly all been increasing their intake in response to present and projected shortages of doctors.

President Nixon's budget for 1974 provided a double blow to the medical schools, for not only did it propose to cut back on spending in 1974, but it also outlined cuts that the Administration intended to make in its own estimates for 1973. The schools are therefore faced with an immediate cut that they had not anticipated, and the prospect of much less money in 1974 than they had bargained for. According to the AAMC survey, the 1974 funds proposed in the Administration's budget are at least 25 per cent less than the schools planned for.

Among the programmes which the Administration plans to cut or abolish, training grants and fellowships, and research grants and contracts from the National Institutes of Health (NIH) constitute the biggest loss to the medical schools. According to the AAMC survey, the 78 medical schools which took part estimated before the Administration's budget was published, that they would receive $599 million from these programmes in 1974, but the budget only contains $465.3 million—23 per cent less than they had planned for. The schools are also suffering from abolition of regional medical programmes and savage cuts in special project grants and general research support grants. One effect of the cutbacks at NIH is that the support of new applications—as opposed to continuing the funding for present projects—will decline by 40 per cent.

According to the AAMC survey, these will be the chief consequences of the cutbacks:

- About 8 per cent of the faculty members and 15 per cent of supporting staff of US medical schools will have to be discharged unless alternative sources of funding can be found
- Curriculum innovations, such as early clinical experience and shortened courses, will suffer
- The abolition of Regional Medical Programs will, according to Dr Cooper, "seriously curtail the ability of the medical schools to become involved in community health programmes"
- One-third of the schools responding to the survey indicated that they may have to reduce the size of the enrolments, and some have already decided to do so
- Some entire research programmes will be stopped.

What are the chances that at least some of the money will be restored to the health budget? There is little doubt that Congress will try to keep the abolished programmes alive, and that it will substantially increase the President's budget request. But the problem is that the White House is still holding the whip hand in budgetary matters. In the present fiscal year, the Office of Management and Budget simply refused to spend several hundred million dollars which Congress had appropriated for various programmes because the Administration wanted to cut down on federal expenditure. Although that precipitated a bitter power struggle between Congress and the White House over which branch of government con-

Shuttle Wins, Science Loses

THE space shuttle easily survived its first Congressional hurdle this year when the House of Representatives last week passed the NASA authorizations bill. An amendment to the bill, seeking to delete the $500 million dollars earmarked for the shuttle, was easily defeated by 95 votes to 20. Congressional critics of the project have three more chances to get rid of the shuttle during the next few months, when the authorizations bill comes before the Senate and when the appropriations bills are considered by each house.

As far as the rest of NASA's budget is concerned, the Committee on Science and Astronautics, which drew up the authorizations bill, reduced the budget request for physics and astronomy by $5 million, and the request for lunar and planetary sciences by $2 million. The money saved from these programmes should be added to the Earth Resources Technology Satellite programme, the committee suggested. The effects of the cuts on space science—if they are agreed to by the Senate—are not yet known, but it is possible that the Atmospheric Explorer satellite may be delayed by about 6 months. But, at least at present, no senior challenge seems to be facing the shuttle.

trols the pursestrings (see *Nature*, **241**, 81 ; 1973), the issue has not yet been resolved. It can thus safely be assumed that President Nixon will veto the HEW appropriations bill again this year if he considers it inflationary or, failing that, the OMB will simply not spend any extra money that Congress may add to the Administration's budget request.

Senator Edward Kennedy, the chairman of the Senate Health Committee, said in a speech on the NIH campus last week that "I will do everything in my power to reverse these short sighted health policies". In particular, he announced that he will soon introduce legislation which will change the statutory basis of the training grant programme, in an effort to force the Administration to keep the programme alive. Bills extending the programmes which the Administration wants to abolish are also in various stages of passage through the Congressional mill.

The Administration's response to the cries of anguish from the medical schools is to stand firm. The general principle that the Department of HEW has been propounding is that the federal government should not be in the business of directly supporting young scientists through training grants, but that the future supply of medical scientists should be left to market forces to determine. At present, the Administration argues that the US is overproducing medical scientists and in testimony before Senator Kennedy's subcommittee, for example, Caspar Weinberger, Secretary of HEW, has called the protestations of the medical schools and scientists "predictable".

PEER REVIEW

Open Attack

by our Washington Correspondent

FEW things affect the biomedical community more directly than the way in which the National Institutes of Health disburses grants and contracts to medical researchers. It is thus not surprising that recent rumours and press reports indicating that the Administration is launching an attack on the very basis of the NIH granting system have been greeted with misgivings and alarm. The subject of the rumours and reports is a memorandum which has been circulating among top NIH officials for the past few weeks. Prepared by the Office of Management and Budget (OMB), the contents of the memorandum have been kept tightly guarded, but last week a copy found its way into the hands of Senator Edward Kennedy and he duly made it public.

It seems clear from the document that the biomedical community has every reason to be alarmed. The focus for the Administration's attack is the peer review system by which grant applications are evaluated, and the memorandum suggests eight ways of "improving" it. They range from total abolition to relatively minor tinkering, but the thrust of the document is towards more targeted research and less of what the Administration has taken to calling "investigator-initiated" research.

The peer review system lies at the heart of the NIH granting process, and although nobody has suggested that it is entirely flawless, it is vigorously defended within NIH and in the rest of the biomedical community. It works like this. A grant application is initially examined by a study group of between 12 and 15 practising scientists working in the same field as the proposal. The study group approves or disapproves the proposal, assigning it a priority rating based on its scientific merit. The next stage is review by a national advisory council, which screens the proposal for policy and programme focus. Each institute of NIH has such an advisory council, consisting of scientific and lay members, and these councils pass recommendations on to NIH staff who in theory retain the final decision on which projects should be funded. In the past fiscal year, some $1,481 million worth of grants and contracts came under the scrutiny of peer review groups in NIH and in the National Institute of Mental Health.

What disturbs the management types in the Office of Management and Budget is that the process seems to give little administrative control to officials of NIH or the Department of Health, Education and Welfare and that makes it difficult to target or direct research. To back up its case against the system, the OMB cites a number of unidentified studies made by the Executive Branch and by Congressional committees. The criticisms which the OMB says have been made of peer review fall generally into three categories. The first is the difficulty in gaining administrative control over the allocation grants, the second is the conflicts of interest which may arise when scientists who are members of study groups "determine the allocation of research funds that they also receive", and the third is that the system defines scientific merit or quality for itself, and is thus "not subject to objective measures of assessment".

Among the eight possibilities for changing the peer review system set out in the OMB memorandum are the following. The most drastic is total abolition of the system and complete reliance on administrative review—the memorandum describes such a step as "the ultimate in return to administrative control". But that would require legislation to remove statutory requirements for national advisory committees to approve grants and contracts. Less drastic are suggestions which would involve retaining the advisory councils, but which would abolish the study groups. In their place, the memorandum suggests that NIH staff could be used for initial review of grant proposals, that consultants could be called in on an *ad-hoc* basis, or that reviews could be conducted by mail by randomly selected anonymous reviewers. To circumvent charges of conflict of interest, the OMB suggests that members of study groups should be changed every two or four sessions, and it also floats the idea of prohibiting members of advisory committees from receiving NIH research funds while serving on the committees or for a time afterwards.

The memorandum is now doing the rounds of NIH officials who will submit their comments to Dr Charles C. Edwards, the Assistant Secretary of HEW for Health. There is little doubt that the institutes will resist fiercely any attempt to scrap peer review entirely, and it seems that in any case there is little chance of that happening. If such a drastic step were taken, it would create much more of an uproar in the biomedical community than even the outcry that has greeted the recent cutbacks in funding and the abolition of training grants.

But, in the context of recent statements by Caspar Weinberger, Secretary of HEW, the trend towards more contract research in NIH, and the general policy of the Administration to concentrate on scientific research likely to have short term payoff rather than on more basic research, some alteration of the peer review system and tighter management control of biomedical research seems clearly on the cards. Weinberger said recently that he wants to "redress the balance" in research funding - "there has been too much emphasis on investigator-initiated research and not enough on directed research".

Members of the biomedical community are concerned, however, that the trend towards targeted research - as exemplified by the cancer programme --is going ahead at the expense of more basic research which could well produce key leads in the drive to understand the mechanism of diseases. The threat to peer review is one aspect of such a trend.

At that meeting, the Council of the Institute of Medicine instructed its President, Dr John R. Hogness, and the President of the Academy itself, Dr Philip Handler, to urge the Administration not to tamper with the peer review system without a thorough study. Congressional backing is also in sight for peer review, for Senator Edward M. Kennedy said last week in a speech on the NIH campus that he will soon introduce a bill to strengthen the system.

NEWS AND VIEWS

Muscular Dystrophy and the Neurogenic Hypothesis

MUSCULAR dystrophy in man has long been regarded as a primary degenerative myopathic process. Histological study of the tissues in such cases coming to autopsy has generally shown that the central nervous system is intact and that the abnormal pathological changes are confined to the voluntary muscles. Recently, however, it has become clear that many patients diagnosed clinically in the past as suffering from muscular dystrophy, particularly of the limb girdle and facioscapulohumeral varieties, may be suffering from a spinal muscular atrophy (the so-called Kugelberg–Welander syndrome). In such cases it is now well recognized that the examination of skeletal muscle specimens obtained by biopsy may reveal so-called secondary myopathic change (Drachman, Murphy, Nigam and Hills, *Arch. Neurol.* (*Chicago*), **16**, 14 ; 1967 ; and Mastaglia and Walton, *J. Neurol. Sci.*, **12**, 15 ; 1971). It has, however, been generally agreed until recently that the most severe form of muscular dystrophy, namely, the X-linked Duchenne variety, which usually produces clinically evident difficulty in walking by the age of 3 years, inability to walk by the age of 10 and death by the age of 20, is still a primary myopathic process. Nevertheless, it is well recognized that the average intelligence quotient in such patients is lower than in a control group of male children of comparable age, and Rosman and Kakulas (*Brain*, **89**, 869 ; 1966) demonstrated neuronal heterotopias in the brains of some such patients at autopsy.

Within the past few years much fundamental work in relation to the pathogenesis of muscular dystrophy has been carried out in animal models, especially mice of the Bar Harbor (129/REJ/dy) strain and in homozygous dystrophic New Hampshire chickens (line 304) (Julian and Asmundson, *Muscular Dystrophy in Man and Animals*, pp. 458–498, Karger, New York, 1963). In 1967, McComas and Mrozek (*J. Neurol. Neurosurg. Psychiat.*, **30**, 526) carried out microelectrode studies from single muscle fibres in dystrophic mice of the Bar Harbor strain and found that approximately one-third of these were functionally denervated. In such mice, electrophysiological studies carried out subsequently by Harris and Wilson (*Nature*, **229**, 61 ; 1971) showed that the number of motor units was reduced, but the surviving units were still of normal size. Furthermore, Jedrzejczyk, Wieckowski, Rymaszewska and Barnard (*Science*, **180**, 406 ; 1973) found in dystrophic chicken muscle a decreased concentration of acetylcholinesterase in the endplates and concluded that a neural factor which determines the synaptic concentration of this enzyme is defective in this disorder.

The relevance of such findings to the pathogenesis of human muscular dystrophy is uncertain because the diseases in such animal models differ in some important respects from the human muscular dystrophies. McComas and Sica (*Lancet*, i, 1119 ; 1970) and McComas, Sica and Currie (*Nature*, **226**, 1263 ; 1970), however, described an electrophysiological technique for estimating the number of functioning motor units in the extensor digitorum brevis muscle of the foot in human patients suffering from the Duchenne, limb girdle and facioscapulohumeral varieties of the disease, and found that the number of functioning motor units was greatly reduced in such cases, even early in the course of the disease. McComas *et al.* suggested that human muscular dystrophy might therefore be caused by a lack of neuronal trophic factor upon which the function and survival of muscle fibres depend.

This concept that muscular dystrophy might well be attributable to a primary neuronal abnormality (the "sick" motor neurone) has become known as the "neurogenic hypothesis". It has been widely challenged, first by histopathologists who have found the earliest pathological changes in human dystrophic muscle to be very different from those of naturally-occurring denervating processes, and by others who have suggested that electrophysiological studies carried out on a muscle (the extensor digitorum brevis) which is not affected early by the dystrophic process, cannot be accepted as being a reliable guide to the nature of the primary aetiological process. Furthermore, Papapetropoulos and Bradley (*J. Neurol. Neurosurg. Psychiat.*, **35**, 60 ; 1972) have found that even in dystrophic mice the total number and morphology of the spinal cord anterior horn cells are normal.

It is true that Bradley and Jenkison (*J. Neurol. Sci.*, **18**, 227 ; 1973) found striking histological abnormalities in the anterior spinal roots of such dystrophic animals, but they gave cogent reasons for suggesting that these neuronal abnormalities are not likely to be of primary aetiological importance. Furthermore, as already mentioned, clinical, electromyographic and histopathological studies in patients previously diagnosed as suffering from limb girdle and facioscapulohumeral muscular dystrophy have shown that many such individuals are suffering from a relatively benign spinal muscular atrophy. It is thus possible that the diagnosis of muscular dystrophy in some of the patients examined by McComas and his colleagues was incorrect, but this criticism seems likely to be invalid in relation to the patients with Duchenne type muscular dystrophy whom they studied, as the latter disease seems still to be a clearly defined and consistent clinical and genetic entity. Thus the role of the motor neurone in the pathogenesis of the muscular dystrophies continues to be a topical and controversial question.

On page 287 of this issue of *Nature*, Kümpel and Dubowitz describe a series of interesting tissue culture experiments in an attempt to clarify the part played by the motor neurone in the pathogenesis of murine muscular dystrophy. They had previously shown (Bishop, Gallup, Skeate and Dubowitz, *J. Neurol. Sci.*, **13**, 333 ; 1971 ; and Gallup, Strugalska-Cynowska and Dubowitz, *ibid.*, **17**, 109 ; 1972) that dystrophic muscle explants obtained from dystrophic mice and from human subjects showed normal growth characteristics *in vitro*, and they were unable to differentiate between normal and dystrophic muscle grown in culture. In the present series of experiments, they cultured muscle cells along with spinal cord explants taken from mouse embryos and found that regeneration and growth of the muscle cells were stimulated by contact with ventral root neurites emerging from the spinal cord explants. Significant differences, however, were found when they cultured normal nerve with normal muscle, normal nerve with dystrophic muscle, dystrophic nerve with normal muscle, and dystrophic nerve with dystrophic muscle. When contacted by ventral root neurites from normal spinal cord, the muscle cells of both normal and dystrophic muscle fused to form new fibres with well-developed cross striations and peripherally-located nuclei. The appearances in muscle coupled with "dystrophic" spinal cord were quite different. The initial cellular regenerative response was followed in most cases by degeneration and disappearance of all muscle elements, leaving only strands of connective tissue. In only three cultures were myotubes formed, and these failed to develop synchronized contractions.

On the basis of their evidence, Kümpel and Dubowitz have reasonably concluded that murine dystrophic muscle behaves morphologically quite normally when regenerating in the presence of normal spinal cord, and, second, that regeneration of both normal and dystrophic muscle is severely abnormal when coupled with spinal cord explants from dystrophic mice. These findings would certainly support the view that in murine muscular dystrophy at least, the primary abnormality lies in the neurone. Whether further investigations, which must be indirect, in view of the impossibility of obtaining viable human anterior horn cells for culture, will confirm that there is a similar defect in the human disease, is, of course, uncertain. But the field is one of rapid development and new information is eagerly awaited.

From a Correspondent

INFECTIOUS HEPATITIS

From Man to Marmoset

from our Medical Virology Correspondent

THERE have been many attempts to propagate human hepatitis viruses outside human volunteers. The observation in 1961–62 of infectious hepatitis (hepatitis A) among human handlers of potential astronaut chimpanzees, and subsequent reports of more than forty small clusters of cases of viral hepatitis in human beings in close contact with young monkeys which had been recently imported, revived interest in experimental transmission studies to non-human primates (*Viral Hepatitis*: *Wld Hlth Org. Tech. Rep. Ser.*, No. 512; 1973). The disease was not, however, transmitted consistently from animal to animal and interpretation was further complicated by spontaneous hepatitis in some monkeys.

Working on the assumption that primates which have little or no contact with man are unlikely to have acquired immunity to the human hepatitis virus, Deinhardt and his colleagues (*J. Exp. Med.*, **125**, 673; 1967) have made use of marmosets, a species of small South American monkey. These monkeys are ferocious, so that contact with man tends to be minimal, and serological surveys of antibody have confirmed that naturally acquired infection to common human viruses is rare. Biochemical and histological changes compatible with hepatitis were found after the inoculation of acute phase serum or plasma from patients with acute viral hepatitis in two species of marmosets, *Saguinus nigricollis* and *S. fuscillois.* Liver damage, without clinical evidence of infection, was produced regularly during five serial transmissions from marmoset to marmoset. Further evidence that marmosets of the species *Saguinus mystax* are susceptible to human hepatitis A, but not to human hepatitis B, was provided by Lorenz *et al.* (*Proc. Soc. Exp. Biol. Med.*, **135**, 348; 1970). Biochemical and histological evidence of liver damage was induced by injecting marmosets with serum derived from a pool of sera obtained from volunteers who were suffering from experimentally transmitted hepatitis A.

Confirmation of these results has just been published. Mascoli *et al.* (*Proc. Soc. Exp. Biol. Med.*, **142**, 276; 1973) inoculated intravenously, during the course of five years, 274 marmosets, chiefly of the species *Saguinus mystax* and a few *S. nigricollis,* with blood from human cases of hepatitis A or hepatitis B. It was found that the indispensable element for the induction of hepatitis in marmosets was the introduction of blood from patients with hepatitis A. As an in-built control, serial passage was carried out in marmosets of a pool of sera obtained before inoculation from marmosets in which the hepatitis A agent was subsequently propagated. Spontaneous hepatitis in marmosets was not encountered.

Blood specimens from five out of seven patients with acute hepatitis A in Costa Rica induced hepatitis in the marmosets. Blood samples taken from patients 22–29 days before the onset of illness yielded negative findings in seventy marmosets. Pools containing samples obtained during the acute illness or during convalescence (29–113 days after the onset of illness) caused hepatitis in 24 of 119 animals. The agent of hepatitis A was passaged successfully four times and it was concluded that the marmoset is highly reliable for detecting, propagating and studying human hepatitis A.

Deinhardt and his colleagues (*Nature,* in the press) have now provided the requisite final piece of evidence by neutralizing the disease in marmosets with serum from convalescing hepatitis patients. The infectivity of the acute phase MS-1 serum from human volunteers and its infectious fractions prepared by density gradient centrifugation in caesium chloride was neutralized with convalescent patient serum, whereas infectivity was unaffected by incubation with human serum albumin or by incubation with preinoculation serum from the same volunteers.

These recent results present exciting advances in the hitherto insoluble problem of the common form of viral hepatitis and the way is now open for investigating the physical and biochemical characteristics of the human hepatitis A virus(es).

FISH STOCKS

Parasites and Whiting

from our Marine Vertebrate Correspondent

THE use of parasites as indicators to supply information on the migrations, past history and even distinctness of stocks of fishes has been a developing and fruitful field of study in recent years. In a new report Shotter (*J. Fish Biol.*, **5**, 185; 1973) applies this technique with conspicuous success to the stocks of whiting (*Odontogadus melangus*) off the Isle of Man. The biology of this fish is in general well known, but its early life history and especially the relationship of offshore stocks and postulated nursery grounds inshore are not firmly established.

It has long been known that the larvae of the whiting are planktonic until about 15 mm in length, when they adopt a commensal relationship with medusae, living under the shelter of the jelly fish—a stage in their life which seems to be essential to their wellbeing. It has been suggested that at the conclusion of this phase they migrate into inshore nursery areas where they feed until they adopt a benthic mode of life and move offshore when they are around 21 cm long.

Shotter's conclusions cast considerable doubt on the validity of this general sequence. He has studied the parasitic fauna of whiting collected in Port Erin Bay, and from two offshore sites in Manx waters. His study has thrown much light on the parasitic fauna and the incidence of the species at different periods of the host's life. This alone represents a considerable advancement to knowledge of this fish and its parasites.

It seems that even the commensal relationship with jelly fishes is not without parasitic hazards for the young whiting. Of the forty-one young fish (15–42 mm in length), thirty were hosts to the copepodids of *Caligus,* which it is believed is itself attracted to the shadow of the medusal umbrella. After about 3 months, however, the association with the medusae ceases and the infestation with *Caligus* declines markedly.

Shotter's main intention was to demonstrate any differences between parasites of populations in different areas and where possible to relate them to feeding habits, physical characteristics of the differing environments and migrations. The comparisons possible between the parasites found in inshore and offshore whiting populations are striking. To cite but two of the ten species of internal parasite listed, the monogean *Diclidophora merlangi* was found in 62% of the offshore fish but in only half this number of the inshore population, and the digenean *Podocotyle atomon* occurred in only 0.8% of the former, but in 47% of the inshore fishes. Another digenean, *Cryptocotyle lingua,* occurred as metacercariae in 28% of the offshore fish but in 67% of those found in inshore waters.

Shotter relates quite convincingly the incidence of these parasites to the local environment of the fish and its food. Thus *Diclidophora* is a direct parasite without intermediate hosts. From the age of about 6 months, the whiting adopts a benthic mode of life and becomes susceptible to infestation from the bottom-living early stages of the monogean. Large infested whiting are found offshore so the continuance of the life cycle is ensured. *Podocotyle,* on the other hand, has as its first host certain mysiid and amphipod crustaceans which are an important food only for the inshore fish, and this parasite is characteristic of fishes which have spent a long time in inshore waters. Similarly *Cryptocotyle* has a life cycle involving the shore-dwelling gastropod *Littorina littorea,* a fish, and as final host one of several species of sea gull; it too is typical of inshore fishes.

The conclusion that Shotter draws from his study of the whiting's parasites is that any migration from the postulated inshore nursery areas to deeper water is probably restricted to a few individual fishes. He suggests that the majority of the inshore fishes may die prematurely as a result of their heavy burden of parasites and increased predation, and that two years is probably the maximal life span of an inshore whiting. It seems from his work that offshore stocks are not recruited from inshore nursery areas as had been thought, a result of some significance in a species which is heavily exploited by the fishing industry.

OIL POLLUTION

Recovery of Salt Marsh

from our Plant Ecology Correspondent

In spite of the abundance of dogmatic assertions regarding the effects of pollution on the environment, which emanate from polluter and conservationist alike, there are surprisingly few experimental or observational data on changes in natural communities which can be directly related to pollution. This applies particularly to plant communities. Where experiments have been conducted, or observations made, they have usually been concerned with the effects of acute, high intensity pollution rather than with those of long-term, low-level chronic pollution.

The Field Studies Council Oil Pollution Research Unit, based at Orielton, Pembrokeshire, has been concerned with the long-term effects of successive oil spillages on maritime communities. It is known that shore communities recover well from single spillages, as is evidenced by the case history of the Torrey Canyon. At Milford Haven, where there is a large oil port, small, successive spillages of oil present a recurrent problem and their long-term effects are unknown.

In a recent report (*Environ. Pollut.*, **4,** 223; 1973), Baker describes the results of four years' observation on experimentally polluted areas of salt marsh in the Milford Haven area. In 1968 three areas of salt marsh which represented successional stages in salt marsh development were selected — low level *Spartina* marsh, mid level *Puccinellia* marsh and high level *Juncus maritimus* marsh. A block of randomized 2 m × 5 m plots was set up in each of the three areas, one being a control, untreated plot and the others receiving monthly sprayings with Kuwait crude oil. Treatments consisted of two, four, eight, or twelve successive monthly sprayings. Changes in vegetation were recorded by means of point quadrats or, in the case of *Spartina,* tiller densities.

The effects of these treatments varied in the different communities. Four or more oilings produced significant reductions in *Spartina* density in the following year. All treatments had recovered within four years, but this was largely because of reinvasion of the plots from surrounding healthy colonies. Successive spillage on a wide scale would probably have a longer-lasting effect: eight or more sprayings in the mid-level salt marsh resulted in a spectacular reduction in the grass *Puccinellia,* which has shown no sign of subsequent recovery. Similar effects were observed in the high-level marsh for *Festuca rubra* and the sea rush, *Juncus maritimus.* In the upper marsh the grass *Agrostis stolonifera* has taken advantage of the niches left vacant by the elimination of these species and has increased considerably.

These data will be of considerable value in predicting the effects of oil spillage on salt marshes. The smaller diversity of vegetation which results from such treatment could lead to lost stability, erosion and the redistribution of large quantities of mud. It would be interesting to know, however, whether the profound effects of eight or more sprayings would have resulted if the treatments had been more widely spread over the four-year period, rather than being concentrated into the first year. It may be that a critical recovery period of more than one month is required for the maintenance of vegetational stability.

DNA Conformation and Initiation of Transcription

When the transcription of DNA by RNA polymerase is initiated the enzyme must bind specifically to the DNA template. Obviously therefore there are two ways in which this initiation step may be regulated, either by changing the structure of the polymerase or by changing the structure of the DNA template. The role of factors such as sigma factor which associate with the polymerase and affect the specificity of initiation has been partially elucidated, but less attention has been given to the effects of changes in conformation of the template DNA on initiation. Travers, Baillie and Pedersen have, however, been investigating this question by measuring the rate of synthesis *in vitro* of *Escherichia coli* ribosomal RNA by *E. coli* RNA polymerase in various conditions which alter the configuration of the DNA template.

As they report in *Nature New Biology* next Wednesday (June 6), using a standard reaction mixture they noticed a lag in the rate of ribosomal RNA synthesis. By varying the order of addition of the components they quickly showed that the DNA template had to be "activated" before efficient transcription began; moreover this activation step seemed reversible. By varying the salt concentration in the incubation mixture, as well as the temperature of incubation and using T4 DNA as a template in some experiments, Travers and his colleagues reached the conclusion that "the substantial changes in both total and rRNA syntheses over a narrow temperature range bear a striking resemblance to a nucleic acid melting curve and suggest that cooperative structural changes in the DNA template may occur". If these changes occur at promoter regions they would, of course, influence the pattern of transcription. In short the activity of a promoter may be controlled directly by controlling its conformation.

PLANT CONSERVATION

Flora Macaronesia

from a Correspondent

THE term Macaronesia is a collective name for the Atlantic Islands including Madeira, the Azores, the Canary Islands and the Cape Verde Islands which have long been recognized by plant geographers as together forming a floristic region characterized by many common elements associated with high endemism; possibly 700 species of flowering plants are endemic to the region.

Unfortunately many of these unique species are now gravely endangered—some are now represented by only a few individuals in a single locality; some may now be extinct. For scientific and historical reasons, their conservation and study have become urgent. Both aspects were discussed in detail at the first international congress "pro Flora Macaronesica" held at Las Palmas, Gran Canaria, in April. Some seventy-two botanists from the Canary Islands, Denmark, France, Germany, Great Britain, Italy, Jordan, Madeira, the Netherlands, Norway, Portugal, Spain, Sweden and the United States participated and provided convincing evidence of the increasing international interest in the Macaronesian flora.

The most important of the decisions made by the congress concerned the preparation of a *Flora Macaronesiaca*, which would provide a synoptic account of the plants of the region, and proposals for the conservation of areas of high scientific importance because the delegates sadly but realistically were well aware that complete conservation is now impossible against the growing demands of tourism, population growth and land speculation, particularly in the Canary Islands. Every island has its own peculiar specialities. A menace to the survival of many endemic species hitherto sheltered by their isolation is the harsh competition of many vigorous introduced alien plants, frequently introduced as ornamental or economic plants and now exterminating the native vegetation.

BIOMATERIALS

Prostheses and Tissue

from a Correspondent

AT the fifth annual biomaterials symposium organized by Clemson University, South Carolina, on April 14, the theme for discussion was prostheses and tissue: the interface problem.

Participants critically scrutinized some of the devices, including buried dental implants and knee and hip joint replacements made from stainless steel, cobalt–chromium alloys and titanium, which are in clinical use on a large scale at present. Numerous patients suffering pain and disability have obtained very considerable short term benefit from these implants, but there is disquiet about the long term results. This point of view was summarized by Dr C. Hirsch (Karolinska Institute, Stockholm) who, in reviewing clinical problems in total hip replacements, said, "Although it is probably an achievement to be able to implant joints, it is obvious that we are far from a clinical situation that can be tolerated".

One clinical problem is infection; another is loosening of the implant with time. The metallic or plastic components used to replace diseased joints are usually fitted to the skeleton with the aid of acrylic resin which is mixed in the operating theatre and allowed to polymerize *in situ*. Acrylic cement has made this type of surgery possible on a large scale, but it is widely recognized that the method is not ideal. Cold-curing acrylic is comparatively weak and brittle and some unchanged monomer, which is toxic, may be present at the end of the polymerization reaction. Patients on the operating table sometimes suffer a sudden drop in arterial pO_2 and cardiac arrest as was discussed by Dr P. I. Kenmore (George Washington University), who recommended that patients should be well oxygenated just prior to and for 15 minutes after the insertion of the acrylic bone cement. The polymerization reaction is exothermic and Dr E. P. Lautenschlager (Northwestern University, Chicago) has measured temperatures up to 70° C at the acrylic-bone interface. Thermal damage and necrosis of tissues, the poor flow and conforming qualities of acrylic dough and its inherent weakness may be why prostheses sometimes work loose.

A third problem has to do with the tissue reaction to minute particles of metallic alloy and their corrosion. Drs D. F. Williams (University of Liverpool) and G. D. Winter (Institute of Orthopaedics, Stanmore) both reported that there are three types of foreign-body particles commonly found in tissues around stainless steel joints and corroded implants: (1) particles of the steel alloy; (2) platy deposits, thought to be corrosion products, which cause a giant cell

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Histone Phosphorylation and Chromosome Structure

A ROLE for histones in the control of genetic activity has been proposed for many years. But histones fell out of favour as control molecules when it was found that their number (and therefore versatility) was small and that their amino acid composition was almost identical in all tissues and, furthermore, in species as far removed as the pea and the cow. More recently, however, interest in histones has been revived with the discovery that these proteins can be enzymatically modified by phosphorylation and acetylation. In *Nature New Biology* next Wednesday (June 6), Louie and Dixon describe studies on the kinetics of phosphorylation and dephosphorylation of testis histones which suggest that such histone modification plays a part in determining chromosome structure.

The kinetics of phosphorylation and dephosphorylation of histone I in trout testis suggest that both "old" and "new" histone I undergo extensive phosphorylation and dephosphorylation throughout the cell cycle. Histones IIb_1 and IV are also extensively phosphorylated, but the kinetics differ from those of histone I. These kinetic differences indicate that specific phosphokinases and phosphotases exist in the cell for each principal histone fraction. Because the half-life of ^{32}P-labelled histone phosphate is 6–8 h, the maintenance of high levels of phosphorylated histones I and IV in trout testis cells over long periods suggests that enzymatic modification of these histones is a continuous balanced process.

Louie and Dixon have proposed previously that the phosphorylation of histones IIb_1 and IV is concerned with the correct binding of these histones to chromatin. They now suggest that the extent of phosphorylation of histone I molecules in a particular stretch of chromatin may determine the degree of supercoiling of the chromosomal fibrils. Fibrils in which histone I is completely phosphorylated would be in the extended form (30–40 Å diameter), intermediate degrees of phosphorylation would allow the extended form to collapse into 100 Å supercoils, and still lower levels of phosphorylation might give rise to coiled supercoils of 200–300 Å diameter. Louie and Dixon have calculated, from their data, the molar phosphate to histone I ratio in the 35 Å, 100 Å and 200–300 Å fibrils and have found that the figures agree well with experimentally determined levels of phosphorylation of histone I in euchromatin and heterochromatin.

The continuous phosphorylation and dephosphorylation of histones I and IV involved in the persistence of the histone in a phosphorylated state suggest that the maintenance of the euchromatic state is an active metabolic process. Regulation of the order of coiling of chromatin fibres by phosphokinases and phosphatases might constitute a coarse control of DNA replication and transcription.

reaction and are fibrotic and necrotizing; and (3) haemosiderin-like granules which have been identified as a mixture of iron oxides and hydroxides.

Titanium has been positively identified at levels in excess of 2,000 p.p.m. in the tissues around some titanium implants. Particles of cobalt-chromium and carbides from the alloy are found in macrophages near cobalt-chromium prostheses. A granulomatous reaction associated with very minute particles near cobalt-chromium total joint replacements was described. The severity of the local tissue reaction depends on the size, shape, chemical nature and particularly on the number of particles present. The otherwise unexplained pain which some patients with prostheses suffer may be related to these foreign-body tissue reactions.

Research is being pursued into the fabrication of materials into which bone will grow and lock the implant into the skeleton. Porous ceramics, titanium, polythene and various forms of pyrolytic carbon are being investigated. Special techniques which have been developed for the histological study of these hard materials were described by Dr J. J. Klawitter (Clemson University).

Dr S. F. Hulbert (Clemson University) and his colleagues have established that the minimum size of interconnecting pores for the ingrowth of bone into calcium aluminate ceramic pellets is 40–100 μm and the material appears to be well tolerated by the tissues. Dr G. D. Winter said that 50 μm was the critical pore size for the induction of heterotopic bone in certain synthetic sponges in the skin of the pig.

Dr L. L. Hench (University of Florida) demonstrated that a cementoid layer forms between surface active glass and glass–ceramic implants and healing bone bonding the two together. The glass surface is purposefully formulated to release sodium, calcium, phosphate and silicon ions. Collagen fibres become incorporated within the 600–1,000 Å cementoid layer and mineralization occurs. In rats, after 28 weeks, the bone to implant bond is stronger in torsion than the bone itself.

A recent report of the clinical use of induced electrical fields to heal a congenital pseudarthrosis of the tibia has stimulated renewed interest in electrical phenomena in tissues. With commendable enthusiasm Dr R. O. Becker (Veterans Administration Hospital, Syracuse) sees the electrical stimulation of hard tissue growth as an alternative to prosthetic devices and he reported success with rabbits in obtaining new growth of articular cartilage over defects on the femoral condyle by implanting bimetallic electrogenic devices.

Next year's symposium will be about the selection of materials for reconstructive surgery.

FEBS

New Foods

from a Correspondent

At the special meeting of the Federation of European Biochemistry Societies (FEBS) in Dublin between April 15 and 19 (see also this week's issue of *Nature New Biology*, **243**, 130; 1973), one of the most interesting contributions was the description by Drs J. F. Connolly (The Agricultural Institute, Dublin), M. Noonan and Professor M. G. Harrington (University College, Dublin) of a physiological mechanism for producing a polyunsaturated lamb chop.

In the body fat of all ruminants (for example, cows and sheep) there is a relatively high concentration of saturated fatty acids which result in part from the hydrogenation of unsaturated fatty acids by microorganisms in the rumen. By bypassing the rumen it might be possible to avoid hydrogenation and produce special products—suitable for patients being treated for coronary heart disease — containing polyunsaturated fatty acids. Indeed in 1961 Ogilvie *et al.* (*Nature*, **190**, 725) avoided the rumen by administering fatty acids directly into the abomasum (or small intestine), and in 1970 a similar result was obtained in Australia by Cook *et al.* (*Nature*, **228**, 178) who fed cows with specially coated seeds as dietary supplements that were rich in polyunsaturates. It was thus possible to produce milk with a high level of such fats and it has been shown that diets containing these products can lower the blood cholesterol.

Dr Connolly and his colleagues exploited a physiological mechanism (the oesophagal groove reflex feeding system (see Ørskov and Benzie, *Brit. J. Nutr.*, **23**, 415; and Lawlor *et al.*, *loc. cit.*, **26**, 439; 1971) by which the suckling ruminant automatically passes food beyond the rumen) to feed lambs substitute diets rich in polyunsaturated fatty acids. A special milk substitute high in linoleic acid was used. The lambs were killed at 36 kg and analyses showed that the body fats of the animals fed the special diet contained ten times as much polyunsaturated fatty acid as the control lambs. Muscle lipids showed a similar trend. It is not without interest that cutlets prepared from these "polyunsaturated lambs" were indistinguishable from those of lambs fed in the orthodox way. The availability of such lambs is commercially feasible so that it should be possible now to formulate special diets aimed at lowering the levels of blood lipids in man. At present only a very limited range of natural appetizing foods are available to patients on cholesterol-reducing diets. Dr Connolly and his colleagues have now begun experiments to produce a veal type calf using a similar feeding system.

Turning from fats to proteins, Professor A. Spicer (The Lord Rank Research Centre, High Wycombe) may well have raised the price of beef in Dublin with his well founded prediction "no beef by 2000". The trouble was that cattle are

New Variant for the Haemoglobin Collection

An interesting new abnormal haemoglobin, with a functional defect, is described by Bromberg *et al.* in *Nature New Biology* next Wednesday (June 6). It has been termed haemoglobin Little Rock (LR), and is unique among the variants so far studied in that it has an anomalously high oxygen affinity without any accompanying diminution in the haem–haem interaction, or any change in the Bohr effect.

A fingerprint analysis establishes that the substitution is glutamine for histidine at position 143 in the β-chains. This is known from X-ray work to be part of the binding site for the cofactor, 2,3-diphosphoglycerate. In the haemolysate the oxygen affinity of haemoglobin LR is greater by a factor of three than that of normal haemoglobin in the adult human.

This difference is not eliminated when the cofactor is stripped off, although the effect of diphosphoglycerate in displacing the oxygen binding curve is appreciably lower than in the normal protein. Evidently, therefore, the affinity for the cofactor is decreased as a result of the substitution by a factor estimated as about 2.5. This effect cannot, however, explain the difference in oxygen affinity.

In an accompanying note, Perutz reports on the consequences of the substitution in terms of the molecular model of haemoglobin. The normal his-143 is not engaged in any interaction with other side chains in either the oxy or the deoxy form, though it does make contact with the cofactor when this is present. A glutamine in the same position in the oxy form is so placed as to make a hydrogen bond with asn-139 in the other β-chain. On deoxygenation, the relative displacement of the β-chains would lead to rupture of these bonds. Thus the oxy-structure in haemoglobin LR is stabilized by the extra hydrogen bonds, and in addition the interaction with the diphosphoglycerate in the deoxy form is weakened because a salt bridge is missing in either β-chain.

Perutz predicts on the basis of these inferences that the dissociation of tetramer to dimer should be inhibited in the liganded forms of haemoglobin LR, and that in the absence of cofactor the increased oxygen affinity should be associated chiefly with a change in the association constant, K_4, for attachment of the last oxygen to the tetramer.

extraordinarily inefficient converters of crop products into protein. Whereas the cow puts on only 1 pound of protein a day, pound for pound growing soya beans put on 100 pounds a day, yeasts put on 100,000 pounds a day and bacteria put on 10^{14} pounds a day.

Dr F. Moran (British Petroleum, Surrey) elucidated the possibility of producing protein foodstuffs from petroleum byproducts. One of the plants run by British Petroleum is turning out 17,000 tons of this food a year, and future plants will have an annual capacity of 100,000 tons. The protein in question has been found to be nutritious and safe and Dr Moran said that it has been cleared as animal foodstuffs in France, Italy, Great Britain, Germany, Denmark and Holland. Clinical tests for human foods were being done and their possible use as additives in baby foods or confectionery was being considered.

On the subject of pollution Dr C. F. Seyfried (Technical University, Hanover) made the prediction that the technique of reverse osmosis would play a very important part in the future treatment of effluents and Dr I. Tookos (Research Institute for Water Resources Development, Budapest), in her presentation on the treatment of organic effluents from the food industry, was quick to point out that biological degradation was preferable.

Dr H. J. Hueck (Central Laboratory TNO, Delft) predicted that the future use of mercury will be severely restricted, and he discussed in particular the use of protective materials containing copper and mercury. "The River Rhine," he said, "carried some 2,900 tons of copper and 70 tons of mercury each year, while Dutch industry—such as metal plating and paint production—spews forth annually 500 tons of copper and 46 tons of mercury."

PROTEINS

Micromethods

from our Molecular Biology Correspondent

THOSE horny-handed toilers of biochemistry who have spent the greater part of their adult life in coaxing tiny cleaning wires through blocked holes in recorder pens, or other occupations of a similar order, might well break out in a craven sweat at the very idea of attempting analytical protein chemistry on extracts from single cells. Nevertheless, gel electrophoresis techniques have, for example, been developed to cope with quantities of protein in the nanogram range, or even below.

A variant that has now been described is isoelectric focusing, done in acrylamide gels of 1 mm or so diameter. Gainer (*Anal. Biochem.*, **51**, 646; 1973) has found it possible to fractionate proteins by this means, and to determine the isoelectric point of a 10^{-10} g sample. As in the usual gel electrofocusing technique, the polyampholyte, which is responsible for establishing the *p*H gradient, is included in the polymerization mixture. The separation, fixing and staining follow more or less the standard large-scale procedures, with certain variations, the trickiest manipulations being those concerned with application of the sample. Results with standard proteins show that the micromethod yields little in the precision of the measured isoelectric points to the standard procedure, using a thousand times more protein. Gainer has already applied the technique to the examination of proteins extracted from single neurones.

The sensitivity of fluorescence, especially of extraneous chromophores chosen for their high quantum yield and capable of attachment to proteins and amino acids, has likewise been put to good use for a number of microanalytical purposes, including, for example, end-group determination. An interesting discovery is now reported by Kinoshita, Iinuma and Tsuji (*Biochem. Biophys. Res. Commun.*, **51**, 666; 1973), who found that the cyclic oligosaccharide, cycloheptaamylose, will sequester dansylamino acids in aqueous solutions, with a huge increase in the fluorescent intensity from the dansyl group. The enhancement is substantially insensitive to added solutes, other than those which themselves form complexes with the saccharide. The sensitivity of the fluorescent assay is such that concentrations of the amino acids after dansylation can be determined at levels well below nanomolar. Kinoshita *et al.* have examined the interaction of the dansyl derivatives of all the common amino acids with cycloheptaamylose, and found that all form 1 : 1 complexes with rather similar free energies, but that the enthalpic and entropic contributions vary somewhat. The small, or unfavourable, entropy changes found for the hydrophobic amino acids can in principle be explained by solvation effects.

Another fluorometric assay for proteins, peptides and amino acids has been evolved by Udenfriend and his colleagues, who discovered that the ninhydrin reaction of primary amines could be modified by the inclusion of phenylacetaldehyde in the reagent, so as to generate a new and blindingly fluorescent chromophore. Since then the nature of this species has been established, and a

Global Tectonics in the Precambrian

DURING the past few years geologists have succeeded in establishing some of the types of geological structure formed early in the history of the Earth. It is, however, far from certain how these structures are related to one another and still less certain what systems of global tectonics operated early in the Precambrian. The successions of volcanic rocks preserved in the Precambrian greenstone belts have no exact analogue today; in some respects they resemble the volcanic materials which accumulate on island arcs but in other respects the rocks can be compared with the predominantly basaltic successions erupted along modern ocean ridges.

Particular attention has been paid to Precambrian anorthosites and it is now clear that in addition to the well known late Precambrian examples, anorthosites were formed rather extensively much earlier in the Precambrian. Two belts in particular have been identified; one extended from North America through Greenland into north-west Europe; the other ranged from Central Africa through Madagascar into India.

Although the exact ages of the anorthosites in these belts are still imprecisely known, they seem to date from a period between 3,500 and 2,500 million years ago. It is known that granitic rocks had already evolved by that period, and this has led to the suggestion that the granitic rocks may represent early continental crust and the anorthositic associations might represent a type of Precambrian oceanic crust. Garson and Livingstone (see *Nature Physical Science* next Monday, June 4) apply this hypothesis to a small area rich in anorthosites which is exposed in South Harris off north-west Scotland; they suggest that the Scottish anorthosites and the associated rocks might represent a piece of oceanic crust thrust into juxtaposition with fragments of a Precambrian continent.

The areas available for study in the Outer Hebrides are so small that it is likely to prove very difficult to arrive at a reasonable interpretation of the large scale structural relations, but it may be possible to do this in Greenland or in the Canadian Shield where more extensive outcrops are present. In this connexion it is interesting to recall that McGregor reported recently that he and colleagues from the Greenland Geological Survey with experience in Western Greenland had arrived at a hypothesis which interpreted the anorthosite rich Precambrian regions in Greenland as primitive oceanic crust.

It will be of interest to see if further work confirms the suggestion arrived at independently by these two groups of workers, and whether future research can establish the relationships of the anorthosites to the greenstone belts and show whether or not these Precambrian igneous associations resemble in any way successions of igneous rocks erupted at the present day.

reactive derivative synthesized. This substance, called fluorescamine, is itself barely fluorescent, reacts more or less instantaneously with amino groups to yield the fluorescent product, and can therefore be used as the basis of an assay (Böhlen *et al.*, *Arch. Biochem.*, **155**, 213; 1973). The fluorescent response is linear with concentration, and the sensitivity is limited by the presence of traces of primary amines as impurities. Under these circumstances the method can be used to estimate 500 ng and up of protein. If, however, impurities of low molecular weight are stripped out by gel filtration in an automated apparatus, this lower level falls by at least an order of magnitude. The response depends of course on the lysine content of the protein, so that for an accurate determination a calibration with the same protein is necessary, but this drawback is, as Böhlen *et al.* point out, no greater than that of the Folin reaction and other colorimetric methods.

In an accompanying report from the same laboratory, Stein *et al.* (*ibid.*, 203) describe the most spectacular application of fluorescamine. If it is used in place of ninhydrin in an amino acid analyser, and the products are monitored by fluorescence instead of absorption, then the sensitivity begins to approach the picomolar range, that is to say 1 μg of protein suffices for amino acid analyses of the highest quality. Hydrolysis is performed in the usual way, and derivatization of the amino acids follows their separation on the ion-exchange columns. Very narrow columns can then be used, with correspondingly greater flow rates, less diffusion, and thus better separation, for a given pumping speed. The reaction is practically instantaneous, and does not need heating.

The possibilities that the method now opens should exercise the minds of many biochemists. Stein *et al.* have already found it possible to determine free amino acids in microlitre volumes of plasma and to perform analyses on minute quantities of proteins in slices of stained polyacrylamide gels, because the ammonia generated during the hydrolysis of the polyacrylamide does not interfere with the fluorescamine reaction, or itself give rise to a highly fluorescent product. A commercial version of the fluorescence-based amino acid analyser is in prospect.

LIQUIDS

Centenary Remembered

from a Correspondent

THE third of a series of conferences on the liquid state, started by the Institute of Physics and Physical Society in 1963, was held at the University of Kent from April 16 to 18, and was on the work of van der Waals and the relationships of this work with contemporary and later developments.

Professor J. S. Rowlinson (Imperial College, London) opened the programme with a paper on the legacy of van der Waals which is to be published in *Nature* in due course. Professor Rowlinson gave a careful account of the history of van der Waals's work on the equation of state, on the critical region and on mixtures, and showed how far it amounted to a synthesis of already existing ideas without detracting in any way from its greatness. Several later speakers returned to this theme of "history repeats itself". For example, Drs A. J. B. Cruikshank and J. W. Goodwin (University of Bristol) showed how van der Waals's ideas are still helpful in interpreting residual functions of liquid mixtures, and Drs Y. de Ribaupierre and F. D. Manchester (University of Toronto) suggested that the hydrogen-palladium system can usefully be compared with a van der Waals gas, the distension of the palladium lattice acting rather like a long-range attraction between the hydrogens.

One of the principal themes was the interplay of different methods. For example Professor J. E. Enderby and his colleagues (University of Leicester) described a comparison of X-ray and neutron studies of strong aqueous solutions of $NiCl_2$, the scattering power of the nickel for neutrons being changed by isotope substitution. Some very interesting conclusions were drawn about this complicated system. Also of interest is the fact that considerable information is obtainable from light scattering (Professor G. Benedek (MIT)) and ultrasonics (Professor D. Sette (University of Rome)) in spite of the fact that the wavelengths are much greater than atomic spacings.

It was also of interest to hear about the state of the art of computer simulation of liquid-like systems, for example, diatomic liquids, fused salts, hard non-spherical molecules, which could certainly not have been tackled until quite recently. Highlights were the invited paper by Dr A. Rahman (Argonne National Laboratory) showing what could be done with only thirty-two "molecules", and the film by Dr R. W. Hockney *et al.* (University of Reading) showing strange "collective" movements in an ionic liquid near its solidification point.

One of the growth points of theory is the study of models of polar liquids. Professor G. Rushbrooke (University of Newcastle upon Tyne) showed how perturbation theory, introducing dipolar interactions into a reference system (for example, the hard sphere fluid), can now be carried through systematically. Dr O. R. McDonald (Royal Holloway College, London) showed the good accuracy now obtainable. Agreement with Monte Carlo calculations is found to be very good except in the notorious region near the triple point which always proves difficult to handle theoretically.

Perhaps the most interesting contribution was the last in the programme. Professor Benedek gave several fascinating examples of the application of optical methods to biological problems. It is now possible, for example, to compare the overall size of a ribosome when it is resting and when it is in the process of synthesizing protein. Laser optics has now attained such refinement that the flow velocity of blood in retinal arteries (about 1 cm s^{-1}) can now be measured by Doppler shift in the frequency of the scattered light.

This most enjoyable conference highlighted the advances that have been made since 1963. There is now a good understanding of "simple" liquids which provides a sound foundation for treating more complicated systems.

Radio Source Associated with OH Source ON-1

WINNBERG and colleagues are presently carrying out a search for compact radio sources close to class 1 OH sources, using the Westerbork Synthesis Radio Telescope and the 100 m at Effelsberg. Their search has proved successful with the first object examined, and in next Monday's *Nature Physical Science* (June 4) they describe observations of a source near ON-1.

The radio source is at

$$\alpha=20\text{ h }08\text{ min }09.8\pm0.1\text{ s}$$
$$\delta=31^{\circ}\ 22'\ 41''\pm1''$$

and the OH source position is

$$\alpha=20\text{ h }08\text{ min }09.8\pm0.5\text{ s}$$
$$\delta=31^{\circ}\ 22'\ 41''\pm15''$$

which, as Winnberg *et al.* point out, seems to suggest that the quoted OH position (from studies at CalTech) is more accurate than the listed errors would imply.

The radio source was unresolved at 5 GHz (WSRT observations) but revealed as a point source at 10.7 GHz (Effelsberg data). Comparison of flux densities at the two frequencies suggests a thermal spectrum with considerable opacity even at the higher frequency. Although only an idealized model can be presented at this stage, the source may, it seems, represent the first stage in the evolution of an H II region.

Most class 1 OH sources are found near large, evolved H II regions because that is where radio astronomers have searched. But it now seems that surveys should be made taking no account of the presence of continuum sources, and that the known sample may be heavily biased by this selection effect.

SELENOLOGY

Lunar Conductivity

from our Geomagnetism Correspondent

IN principle, it is possible to determine the lunar electrical conductivity by measuring the electromagnetic response of the Moon to variations in the magnetic field of the solar wind. In the indirect method, the first step is to calculate the response of a theoretical conductivity distribution and compare it with the response obtained experimentally from magnetometer records. The conductivity structure is then adjusted until the observed and calculated responses agree as closely as possible. Using this technique with data from a magnetometer on the lunar surface and a second magnetometer on the lunar satellite Explorer 35, Sonett *et al.* (*Nature*, **230**, 359 ; 1971) first obtained an irregular conductivity-depth distribution, the chief features of which were a relatively low surface conductivity and a highly conducting zone at a depth of about 240 km. But Kuckes (*Nature*, **232**, 249 ; 1971), using the same data, came up with a quite different distribution comprising a uniformly conducting inner sphere and a non-conducting outer shell about 160 km thick. Yet both models agreed with the experimental data to within about one standard deviation.

As Hobbs (*Earth Planet. Sci. Lett.*, **17**, 380 ; 1973) now points out, if at least two quite different conductivity models can be consistent with the same data, it is reasonable to ask what any model derived from the data means. Accordingly, he has carried out an analysis of the lunar conductivity problem in the light of recent work by Backus and Gilbert (see, for example, *Phil. Trans. Roy. Soc.*, **A266**, 123 ; 1970) in which the general question of determining infinite-dimensional models from a finite quantity of data is examined. In the case of the Moon, the infinite-dimensional model is the lunar electrical conductivity at all radii, and the finite data are provided by the measured electromagnetic response at various frequencies and the associated estimates of error. Similar considerations would also apply to the Earth, of course ; and because data on the response of the Earth to external magnetic field variations are far more abundant than those relating to the Moon, it is instructive at the outset to note the result of applying the Backus–Gilbert ideas to the problem of terrestrial conductivity. As Parker (*Geophys. J.*, **22**, 121 ; 1971) has shown, the fact is that in spite of the relatively abundant terrestrial data, the conductivity distribution in the Earth is still poorly defined. This emphasizes the importance of determining just what information the more fragmentary lunar data can give before non-unique conductivity models come to be accepted uncritically as the basis of other investigations.

The specific aim of Hobbs in his first-order analysis was to determine the fineness of detail that can be resolved in the lunar conductivity profile using the available experimental response data. To set a limit on this fineness of detail, he assumed that the data are error free ; in other words, if any errors are admitted, the uncertainty in the derived conductivity will be increased. The end product of the analysis is then a smoothed conductivity-depth distribution for each model originally found to be consistent with the data, the models examined being those of Sonett *et al.* and Kuckes.

When the smoothed conductivity profile from Sonett's irregular distribution is compared with that from Kuckes's two-layer distribution, both profiles are found to exhibit little variation in conductivity over the approximate depth range 170–700 km (no estimates are possible below 700 km because there is no field penetration for the frequencies considered) but a lower conductivity in the upper 170 km. In other words, the finest detail resolvable is that of a two-layer model in which the surface layer has a lower conductivity ; and the irregularities in the Sonett distribution, such as the high conductivity zone at 240 km, represent greater detail than the data can sustain. This is not to say that such irregularities do not exist, but only that they are not derivable from a first-order analysis of the available data. Moreover, nor are the smoothed profiles necessarily the most probable or best-fitting distributions ; they are merely the smoothest distributions consistent with the data.

The conclusion is, therefore, that in spite of their obvious differences, the models of Kuckes and Sonett *et al.* really only give the same information about the distribution of the Moon's gross conductivity. In view of the fact that both models are based on the same data, this is hardly surprising. But what Hobbs does consider "a little surprising" is the persistence of models containing a thin, highly conducting layer near the Moon's surface. As he has shown, such models are consistent with the data but not derivable uniquely from them ; they are the product of inadequate data rather than evidence for the idea of "frescons". Hobbs is thus, in effect, warning against taking the irregular distributions too seriously at this stage and strongly implies that, for example, the lunar temperature profile recently inferred by Duba *et al.* (*Earth Planet. Sci. Lett.*, **15**, 301 ; 1972) on the basis of the Sonett model is premature.

Sulphur Content of the Earth's Core

EVER since it was discovered that the density of the Earth's outer core is apparently 8–10% lower than the density of pure iron, there has been considerable discussion about the nature of the light component alloying with the iron. Perhaps the most popular contender is silicon, although another possibility—and one that has been receiving increasing attention in recent years—is sulphur. Because of the interest in sulphur, King and Ahrens have carried out a series of shock wave experiments on pyrrhotite to determine whether the equation of state is consistent with the seismic data and (in connexion with a forthcoming report by Usselman) whether the sulphur content inferred is in agreement with the Fe-FeS eutectic composition at core pressures. The results of these experiments are reported in *Nature Physical Science* (June 4) next Monday.

King and Ahrens have obtained shock pressure-density data over the pressure range 198–405 kbar using six pyrrhotite ($Fe_{0.9}S$) samples machined from a single crystal, and find that the slope of the resulting Hugoniot is significantly greater than that to be expected on the basis of the ultrasonic data. They conclude from this that the pyrrhotite in the pressure range 289–405 kbar is in a shock-induced high pressure phase and that at the lowest pressure considered the pyrrhotite is of mixed phase, being only partially transformed to the high pressure phase. The estimated zero-pressure density of the new high pressure phase lies in the range 5.2–5.6 g cm^{-3}, which compares with the 4.6 g cm^{-3} of the original material.

By extrapolating the data for the high pressure pyrrhotite phase to the pressure and likely temperature (about 3,500 C) in the outer core (which assumes, of course, that no further phase changes occur) and comparing the result with corresponding pure iron data, King and Ahrens estimate that the core density at the core-mantle interface (9.94 g cm^{-3}) is achieved by the presence of 26–31% of the high pressure $Fe_{0.9}S$ phase or 10–12% (weight) of sulphur. Further extrapolation throughout the outer core introduces greater uncertainties but, taken at face value, implies a sulphur content of 10–15% to be consistent with the observed profile of pressure-density data.

These new estimates of the supposed sulphur content of the outer core agree well with the values of 10%, 15% and about 9–12% obtained by other workers using different methods.

Tree-ring Calibration of Radiocarbon Dates and the Chronology of Ancient Egypt

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The historical calendar of Ancient Egypt is the only independent chronology for testing the bristlecone pine calibration of the radiocarbon time scale from 3000 to 1800 BC. Here a statistical approach is used to compare the functions relating the Egyptian historical dates to the corresponding radiocarbon dates and the bristlecone pine tree-ring dates to the corresponding radiocarbon dates, and it is concluded that the calibrated radiocarbon dates for Egypt do not differ significantly from the historical dates in the time period considered, although the errors associated with these preclude a precise comparison. The accord gives qualified support for both systems and invalidates some published objections to the use of a calibrated time scale in prehistoric archaeology.

THREE radiocarbon laboratories, at La Jolla, Philadelphia and Tucson[1-3], have obtained radiocarbon dates over the past decade for specimens of bristlecone pine already dated dendrochronologically[4], thereby allowing the "correction" of the radiocarbon scale. The calibration curve produced by Suess[5] is now widely used for this purpose. Its precise form has been questioned by some workers[6], especially the validity of the "kinks" or short-term fluctuations in the curve, and the nature of these short-term fluctuations in the atmospheric concentration of radiocarbon remains a problem[7]. In some quarters doubt has been expressed over the reality and magnitude of the first order deviation itself[8].

The historical calendar of Ancient Egypt has been used several times for checking the value of the radiocarbon method[9] although more recently the divergence between the radiocarbon dates and the historical chronology has given grounds for disquiet[10,11]. The discrepancies are reduced by applying the bristlecone pine calibration to the Egyptian radiocarbon dates[12-15], but some scholars have suggested that this now makes these Egyptian dates systematically too old instead of too recent. One worker has indeed advocated the calibration of radiocarbon dates from archaeological samples by reference to the Egyptian dates alone and without regard to the bristlecone pine[8,16].

Sufficient data are available to permit a statistical investigation of both problems, taking into account the experimental errors in the radiocarbon determinations. The time range selected covers the period from the beginning of the Egyptian historical chronology (about 3100 BC) to the first secure and astronomically verifiable Egyptian historical date (1872 BC).

Calibration Function

Any calibration curve based on tree rings is derived ultimately from the analysis of a series of tree-ring-dated wood samples, where each sample has both a tree-ring date (generally assumed to establish a date in calendar years with a probable error of less than two or three years), and a radiocarbon date with its attendant standard error arising chiefly from counting problems. Similarly, the historical data used for any check on the dendrochronological calibration arise from a series of samples each having, as well as a radiocarbon date (with standard error), a date in calendar years assigned on the basis of its observed archaeological context and the appropriate historical calendar.

Here we investigate the proposition that the calibration relationship connecting radiocarbon dates and their corresponding "true" dates is the same in both sets of data. If this proposition is correct, it would lend support to the use of bristlecone pine radiocarbon dates to calibrate radiocarbon dates relevant to prehistoric material in general.

Our method assumes that the calibration relationship is of the form $y=f(x)+\varepsilon$ where y denotes the radiocarbon date of a sample, x its corresponding "true" date (as determined dendrochronologically or archaeologically), ε the random measurement error in the radiocarbon date, and f the unknown "calibration function". This function is approximated by fitting a suitable smoothing function g, using a weighted least-squares procedure. The hypothesis is then tested by standard statistical methods, essentially by comparing the goodness-of-fit of a single smoothing function g fitted to the whole data, with that of two smoothing functions of the same type fitted separately to the two sets of data. If a common curve fits just as well as two separate curves, one may conclude that there is only one calibration curve for both the Egyptian and bristlecone pine data. As the choice of type of smoothing function may be crucial, this method has the important advantage of enabling several possible such functions to be easily compared.

Earlier workers have approached this problem by fitting a smoothing function g to the bristlecone pine data alone, and then matching the observations from individual Egyptian samples against that curve. This can be done in two ways: (a) by comparing, for each Egyptian radiocarbon date, the corresponding calendar date(s) as calibrated against *g* with the assigned historical date for that sample; (b) by comparing, for each Egyptian historical date, the corresponding radiocarbon date as predicted by *g* with the date actually observed (see Fig. 1). In both cases it must be remembered that the smoothing function *g* is only an estimate of the calibration function *f*, and therefore has its own associated standard error.

In such a procedure method (b) is preferable to (a) for two reasons. (i) It avoids the possibility of several calendar dates corresponding to a single radiocarbon date if there are "kinks" in the calibration curve (see Fig. 1). (ii) The comparison of predicted and observed radiocarbon dates is preferable to the comparison of predicted and observed calendar dates, for it is the radiocarbon dates which are subject to random errors of known magnitude. The errors associated with the tree-ring dates themselves are thought to be small[4], whereas those involved in assigning precise historical contexts of samples of Egyptian material are difficult to quantify or treat statistically.

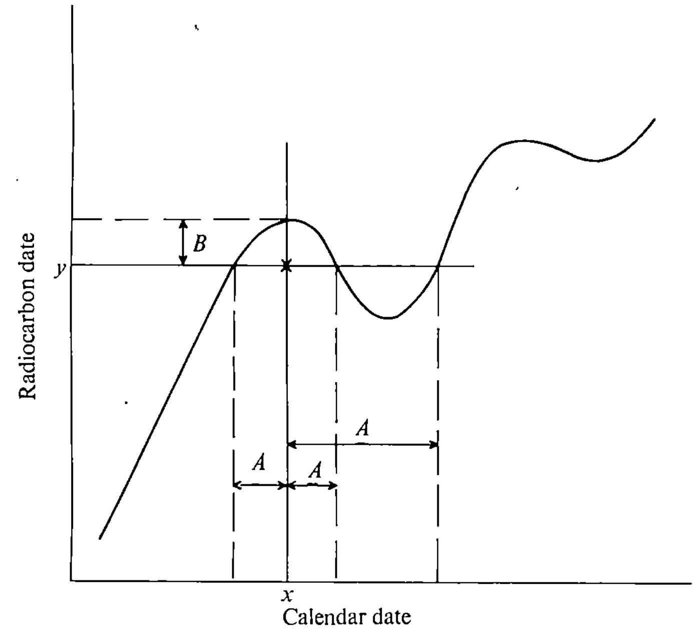

Fig. 1 Methods (a) and (b) of matching a typical Egyptian sample against a fitted calibration curve. Method (a) considers the discrepancies *A* between the estimated and known calendar dates; method (b) considers the discrepancy *B* between the observed and predicted radiocarbon date. ×, Plotted point corresponding to Egyptian sample; ——, calibration curve.

Recent workers[12,14] using method (a) have not correctly allowed for the standard error of a calibrated radiocarbon date. Such a standard error depends not only on that of the radiocarbon date itself, but also on the nature and precision of the smoothing function near the point of calibration. An important disadvantage of Suess's calibration curve[5] is that, as it is not determined by explicit reproducible methods, its precision cannot be reliably assessed.

A crucial problem is the choice of type of smoothing function *g*. Among those already applied to radiocarbon determinations for samples of known age are linear functions[17], periodic functions[18], polynomials[19], and the visual fitting of a subjectively-drawn curve[5]. There is no universally valid criterion for preferring a particular approach, and indeed it is relevant to consider some of the general criteria which might be thought desirable in a smoothing function.

(1) It should be derived by a reproducible and non-arbitrary method.

(2) There should be an objective method of testing whether the curve is an adequate fit to the data.

(3) The fitted smoothing function should in fact be an adequate fit to the data.

(4) There should be an objective estimate of the error in the fitted smoothing function.

(5) The smoothing function need not, however, possess the same general properties (such as continuity, smoothness) as the calibration function, although they may be desirable.

The choice of smoothing function will naturally depend on the purpose of the smoothing. In the case presented here, the smoothing is intended to facilitate the comparison of the Egyptian and bristlecone pine dates. We are not claiming to have produced a universal all-purpose calibration curve relevant to this time period. The production of a suitable calibration curve for use by archaeologists is a related problem at present under investigation. The physical background may well require that the calibration function *f* should have a bounded continuous first derivative, while the smoothing function *g* need not necessarily be constrained to have this property. For the present purpose, a discontinuous function, such as a series of unconnected straight lines, may be statistically convenient and quite valid, even if it would not be satisfactory as a calibration curve.

Evaluation of the Data

The data for our analysis consisted of the assigned historical dates, and the corresponding radiocarbon dates with their associated standard errors, for fifty-two Egyptian samples relevant to the time period 3100–1800 BC (calendar years), comprising fifty-one radiocarbon dates given by Berger[20] and Edwards[21], with the subsequent corrections[22] noted, and one extra sample (BM 236) found to be paired with UCLA 1208 (ref. 22). We also used the tree-ring date and radiocarbon date corresponding to each of fifty-seven specimens of bristlecone pine relevant to this time period, obtained from the list set out by Houtermans[18]. These specimens were dendrochronologically dated by the Laboratory of Tree-Ring Research, University of Arizona, and their radiocarbon dates determined by Suess at the La Jolla laboratory. Concerning the standard errors of these radiocarbon measurements, Houtermans[18] states only that they range from 4 to 7 per mil, and so we decided, in the absence of more specific information, to assign to these radiocarbon dates a common standard error of 5.5 per mil or 45.5 yr, the midpoint of the range.

In constructing a calibration curve, or comparing one proposed curve against another, the magnitude of the errors of measurement is of course important, as this determines how well a given smoothing function *g* approximates the unknown calibration function *f*. So, before fitting any smoothing function, it is essential to verify the quoted measurement errors whenever possible.

The standard error of 45.5 yr assigned to the La Jolla radiocarbon dates is at least partially verified by the estimated error variance, namely 2,263 on eleven degrees of freedom, obtained by Clark and Renfrew[17] by considering the differences[23] between replicate radiocarbon determinations carried out by the La Jolla laboratory on samples of wood from Auvernier, Switzerland. These Auvernier samples, however, were not of wood of the bristlecone pine, and so the quoted variance may not necessarily represent the precision of the La Jolla laboratory with bristlecone pine samples.

As many of the Egyptian samples were divided and analysed by both the BM and UCLA laboratories, the comparison allows the evaluation of interlaboratory variations, and hence of the published standard errors which the laboratories associate with their determinations.

Examination of the twenty-five paired differences so obtained showed that there was no systematic difference between these laboratories, but that the paired differences showed significantly greater variation than that expected due to measurement errors, the corresponding χ^2 value being significant at the 0.2% level. This implies that either the real measurement errors are greater than believed, or that there were some gross (non-counting) errors in some of the dates, or both. The standard errors of measurement would have to be 20% to 40% greater than the quoted standard errors in order to explain the full observed discrepancy.

McKerrell[8] has also concluded that there is no evidence of a systematic difference between these laboratories, but failed to note the excessive variation in the paired differences. The inclusion of two pairs omitted by McKerrell and the use of a more sensitive test does, however, document the excessive variability in the paired differences.

Some impression may be gained of the reliability of the ascription to the samples of a historical context by the Egyptologists concerned, together with the variation in the age of the samples when buried, by comparing the measured radiocarbon dates of samples from different archaeological contexts reported as approximately contemporary. Several analyses are available from different contexts assigned archaeologically to the same period. If these archaeological assignations are correct, the differences in the radiocarbon dates for them should be no greater than would arise from the laboratory measurement errors alone. The question here is not simply the major one of the validity of the Egyptian historical calendar[11,21,24] but the more practical one of the precision with which these historical dates may be assigned, assuming for the moment their correctness, to the specific samples in question, on the basis of their stratigraphic contexts.

Ten groups of dates were considered, where all the samples within each group were supposedly of the same historical date, and their radiocarbon dates determined by the same laboratory. In three of these groups, the range of the corresponding radiocarbon dates was significantly greater than could be explained by measurement errors. Table 1 summarizes the results of the ten significance tests performed using Table 22 of Pearson and Hartley[25].

Table 1 Significance of Range of Radiocarbon Dates of Supposedly Contemporaneous Egyptian Samples

Historical date	BM	UCLA
3025 BC	* (4)	—
3000 BC	—	ns (2)
2950 BC	ns	—
2650 BC	† (4)	ns (2)
2600 BC	ns (3)	—
2335 BC	ns (2)	ns (2)
2000 BC	ns (5)	‡ (4)

—, No dates available; ns, not significant at 5% level; *, significant at 5% level; †, significant at 0.1% level; ‡, significant well beyond the 0.1% level (about 0.001%). Number in parentheses is number of samples in each group.

These results imply (i) that some of the measurement errors are greater than indicated by the laboratories, or (ii) that certain groups of samples are not in fact contemporaneous (which was assumed in these tests), or (iii) there are gross errors in some of the radiocarbon dates. If the measurement errors were increased by 40%, as suggested previously, the two most significant groups would still show significantly large ranges. The observed discrepancies cannot be explained simply by increasing the standard error of measurement to the extent suggested by the interlaboratory comparison. But the four samples given an historical date of 2650 BC and the five corresponding to 2000 BC were of different materials (such as wood, linen, reed), and these samples might not be of identical age, even when they were buried.

It is concluded that some of the information concerning the fifty-two Egyptian samples is grossly in error: either some "true" dates, or some radiocarbon dates, or their associated standard errors. This has not previously been demonstrated by a systematic statistical analysis, although Michael and Ralph[26] and Säve-Söderbergh and Olsson[27] have used qualitative comparisons to draw similar conclusions, the latter pointing to uncertainties in some cases about the archaeological date. There does not seem to be any independent objective method of deciding, on the basis of existing data, which pieces of information are wrong and by how much. We suspect that in some cases difficulties in stratigraphic interpretation have led to error. Admittedly, the assigned Egyptian historical dates are not claimed to be exact, but there seems to be no objective way of quantifying their lack of precision.

These results alone show clearly that the attempt to use the Egyptian historical dates as the basis for a "calibration" of the radiocarbon chronology, quite independent of the bristlecone pine dendrochronology[16], is not well founded.

These discrepancies make the Egyptian data less suitable for a check on the bristlecone pine calibration than might be hoped. Both Berger[20] and McKerrell[16], however, have based comments on the calibration upon an examination of these Egyptian data, and it is important to consider such claims. In what follows analyses are therefore conducted on the provisional assumption that the assigned historical dates for the Egyptian samples are without error, and that the discrepancies observed are simply the result of high measurement errors of their radiocarbon dates. Although, as already noted, this assumption is not strictly warranted, it should not affect the conclusions unless the historical dates are very seriously in error, as the F-values in Table 2 are not near the critical values.

Statistical Analysis

The problem of testing the hypothesis of a common calibration curve for the bristlecone pine and Egyptian data can be recognized as a special case of testing a sub-hypothesis in a general linear model, for which standard statistical techniques are available[28]. In order to apply these techniques, it was necessary to assume, at least provisionally, that the calendar dates assigned to all Egyptian and bristlecone pine samples were without error, and that the errors of measurement of the radiocarbon dates were normally distributed. It was also assumed that the standard errors of all radiocarbon dates used were equal to k times the corresponding reported standard errors. The factor k, initially unspecified but later estimated from the data, allows for possible underestimation by all three laboratories of their overall measurement error. As has been noted elsewhere[29,30], the reported standard error refers only to "counting error", which is only part of the overall measurement error. The preceding analysis of paired differences indicates that such underestimation of the real measurement errors could be substantial.

Two types of smoothing function g were fitted to the data over the range 3100–1800 BC, using the method of weighted least-squares to allow for the differing precision of the radio carbon measurements. As the weights used in this procedure depend only on the relative magnitudes of the standard errors of the radiocarbon dates, the weights can be chosen without knowing the actual value of the factor k. First, a single mth degree polynomial was chosen, giving in fact fourteen curves corresponding to the various values of m up to a maximum of

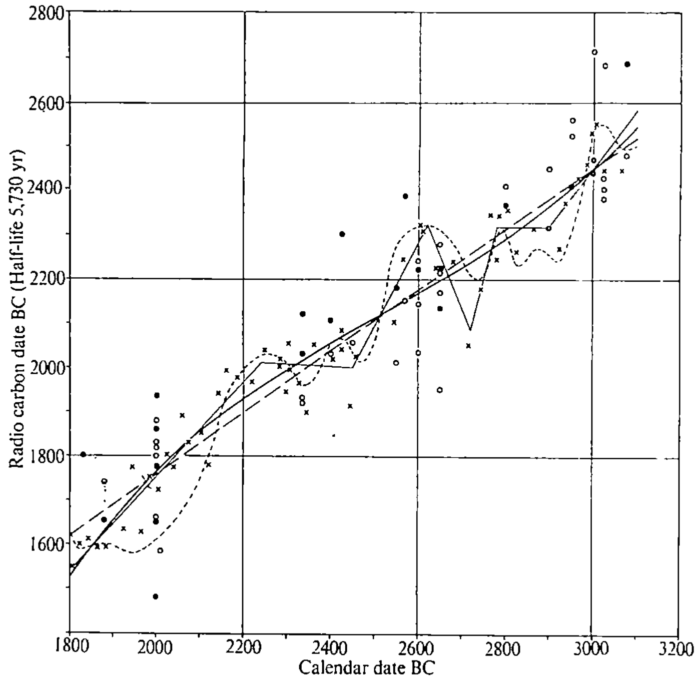

Fig. 2 Relationship between radiocarbon dates and calendar dates, showing the curve published by Suess - - -, and three of the smoothing functions fitted by weighted least-squares: — —, straight line; ——, fourth-degree polynomial; ——, piecewise linear curve. Plotted points represent data used in this analysis: ×, bristlecone pine (LJ); ○, Egyptian (BM); ●, Egyptian (UCLA).

14 imposed by the limits of computer accuracy. Second, *g* was assumed to comprise seven straight lines constrained to be continuous at the six corresponding join-points (see Fig. 2). The number of line-segments was restricted to seven because of computer capacity and the location of the resulting six join-points was estimated statistically without reference to Suess's curve.

Amongst the Egyptian data, in most cases there was more than one radiocarbon date corresponding to each of the eighteen distinct historical dates. For the purposes of curve-fitting, each group of radiocarbon dates corresponding to each historical date could be replaced by a weighted mean radiocarbon date. The residual variation after fitting any smoothing function *g* to these data can therefore be partitioned into two components. The first component involves the deviations of the weighted groups-means from the fitted function, whereas the second component represents the within-groups variation for these radiocarbon determinations of supposedly contemporary Egyptian samples. Being the same for all fitted functions, this second component measures the intrinsic variability of the Egyptian data, which no amount of curve-fitting can reduce.

The corresponding analysis of variance is given in Table 3 for the case where the function *g* is simply a straight line. The weighted least-squares procedure is exactly equivalent to transforming the data so that the transformed radiocarbon dates have a common error variance and then performing a standard least-squares analysis of the transformed data. In this particular case, the weights were chosen so that this common error variance was equal to $(45.5\ k)^2$. Item *e* in Table 3 is an unbiased estimate of this variance, even if the fitted straight line is not an adequate fit to the data and even if our hypothesis is incorrect. It follows then that the factor *k* must be at least 1.5 and possibly 1.8, implying that the true standard errors of the radiocarbon dates must be at least 50% and possibly 80% greater than the quoted standard errors. This reinforces the doubts expressed earlier concerning the validity of the Egyptian data.

In Table 3, comparison of items *d* and *f* with *e*, using the appropriate *F*-tests, shows that a straight line is in fact an adequate fit over the time range to both the Egyptian and bristlecone pine data separately. Moreover, comparison of items *g* and *e*, yielding the low *F*-value of 1.08, indicates that a single straight line fits the combined data just as well as these two separate lines. In other words, the hypothesis of a common calibration curve (in this case, a straight line) is well supported.

Table 2 Comparison of Goodness-of-Fit of Various Smoothing Functions Applied to Egyptian, Bristlecone Pine and the Combined Data

Type of curve	Bristlecone pine data Residual s.s.	m.s.e.	Egyptian data Residual s.s.	m.s.e.	Combined data Residual s.s.	m.s.e.	*F*-value	Degrees of freedom
Linear	310,319.72	75.11	337,186.78	82.12	661,938.08	78.65	1.08	2,34
Quadratic	291,545.86	73.47	333,640.54	82.51	657,691.58	78.76	1.63	3,34
Cubic	266,432.82	70.90	333,599.58	83.37	627,952.14	77.33	1.05	4,34
Quartic	266,371.64	71.57	320,793.39	82.62	625,044.44	77.52	1.14	5,34
Eighth degree polynomial	214,232.53	66.80	298,367.62	83.30	602,018.40	77.58	1.49	9,34
Fourteenth degree polynomial	167,840.19	63.21	270,739.99	85.54	543,762.94	76.05	1.05	15,34
Seven connected lines	151,717.62	55.64	300,483.77	82.64	539,256.58	73.07	1.64	8,34

Residual s.s., weighted residual sum of squares; m.s.e., mean-square-error. *F*-value is the value of the standard *F*-statistic for testing the hypothesis of a common calibration curve of the given type. Items *a*, *b* and *c* of Table 3 are given in the first, third and fifth columns respectively.

Similar tables of analysis of variance may be constructed for each fitted smoothing function, simply by substituting the value of items a, b and c given in Table 2, and using the well-known structure of analysis-of-variance tables. The resulting F-values for testing the hypothesis of a common calibration function are listed in Table 2. In all the cases considered, this hypothesis was supported, as testified by the low F-values. Table 2 also gives a comparison of the goodness-of-fit of the various smoothing functions when fitted to the combined data or to either the bristlecone pine or Egyptian data separately.

If the assigned historical dates of the Egyptian samples were not correct, then the various F-statistics would have only an approximate F-distribution, rather than the exact F-distribution assumed in the preceding significance tests. But, provided these dates are not very seriously in error, our conclusions remain the same, because the computed F-values are comparatively distant from the critical values. As there is no objective measure of the precision of these estimated dates, no completely objective statistical test of the hypothesis is possible, using the given data. Method (a) seems to overcome the problem by calibrating the Egyptian radiocarbon dates using a calibration curve based on bristlecone pine observations only, but there is then no objective way of comparing those calibrated dates with the assigned historical dates. Some assumption concerning the precision of these dates is thus necessary for any analysis, and our assumption facilitates the comparison of several smoothing functions. The remaining assumptions are likely to be at least approximately correct and, in any case, the analysis would be only marginally affected by small departures from them.

Table 3 Analysis of Variance due to Fitting Linear Smoothing Functions

Source of variation	Degrees of freedom	Sum of squares	Mean-square
Deviation of weighted group means from Egyptian line	16	111,724.76	6,983 *d*
Within-groups (Egyptian)	34	225,462.02	6,631 *e*
Total residuals from Egyptian line	50	337,186.78 *b*	6,744
Residuals from bristlecone pine line	55	310,319.72 *a*	5,642 *f*
Difference between lines	2	14,431.58	7,216 *g*
Residuals from common line	107	661,938.08 *c*	
Regression due to common line	1	5,895,145.74	
Total	108	6,557,083.82	

Validity of the Time Scales

Detailed analysis of the Egyptian data shows that, if major experimental errors be discounted, either the standard errors associated with the radiocarbon dates have been grossly underestimated, or some of the calendar dates assigned to samples on stratigraphic evidence are seriously in error, or both. It would therefore be ill-advised to use the Egyptian data to construct a calibration curve independent of the bristlecone pine chronology, although this has been suggested by McKerrell.

These deficiencies in the Egyptian data unfortunately preclude any completely objective statistical analysis, or any exact comparison of the two time scales. We claim, however, that our analysis is the most precise comparison possible in the circumstances. A more exacting test of the comparability of the two data sets could only be obtained with better data, and in particular better Egyptian data.

Nonetheless, although the comparison is not as precise as is desirable, the available information indicates that the hypothesis of a common calibration curve for the bristlecone pine and Egyptian data over the time period 3100 to 1800 BC is not contradicted, and suggestions to the contrary are not well founded. The conjunction of the bristlecone-pine-calibrated Egyptian radiocarbon dates and the historical dates for Ancient Egypt from 3100 to 1800 BC carries with it the implication that, within the error limits discussed, both chronological systems are correct. It seems unlikely that the Egyptian historical calendar, as reconstructed by Egyptologists today, and the bristlecone pine calibration of radiocarbon dates should be in error in precisely the same way over this range, so as to yield closely comparable yet erroneous calibration functions.

Our analysis thus lends support to the general validity of both the radiocarbon calibration and the Egyptian historical chronology. Further accurate work on Egyptian material is still required to reduce the level of error and thus to make the comparison more sensitive. But the present harmony gives some grounds for optimism concerning the validity of the bristlecone pine calibration as applied to prehistoric studies in general.

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Turning the Left Cheek

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This article explores the fact that portrait painters have tended to paint the left cheek rather than the right one.

THE "likeness" of a person's face is seldom well conveyed when the profile is not shown, and artists have usually portrayed their subjects facing to one side. Which side? Why should it matter: the right cheek is generally as representative of the man as is the left? Yet analysis of actual portraits shows there to have been a consistent tendency to paint the left cheek rather than the right. Moreover the tendency is significantly more marked both in portraits of women than of men and in portraits showing the subject's body than in those showing just his face.

The material for this study was 1,474 painted portraits, produced in Western Europe from the sixteenth to the twentieth centuries. The sources of these portraits were: the National Portrait Gallery, London (571 portraits), a textbook on Elizabethan and Jacobean portraiture[1] (338 portraits), the portraits and portrait miniatures of the Fitzwilliam Museum, Cambridge (231 portraits), a miscellaneous collection of art books belonging to the first author (291 portraits) and the exhibition at the National Portrait Gallery entitled "The Masque of Beauty" (August 1972) (43 portraits).

All portraits showed a single person only. For each the following points were noted: the sex of the subject; the side of the subject's face which was turned towards the viewer; and the amount of the subject's body which was visible in addition to his head. Rare cases of full-face portraits were excluded from the study.

Table 1 Data by Sex

	Left	Right	% left	P
Men	524	408	56	<0.001
Women	376	175	68	<0.001

Of the 1,474 portraits 891 showed more of the left cheek than the right. This 60% bias to the left is highly significant ($P<0.0001$ on a χ^2 test). Table 1 shows the data broken down by the sex of the subject. Although the left-cheek bias is significant in portraits of both men and women, the bias in women's portraits (68% to the left) is much greater than in men's (56% to the left), the difference being significant at the 0.001 level.

Analysis of the data in terms of the amount of body visible is shown in Table 2. Here the portraits have been subdivided into two groups, "head only" or "head and body", according to whether or not any of the body below the shoulders was portrayed. For both men and women the left-cheek bias is much stronger when the body below the shoulders is included in the picture. Taking both sexes together, the difference between the "head only" group (51% to the left) and the "head and body" group (65% to the left) is significant at the 0.001 level.

Several explanations may be offered for these findings. For instance, there might be bias in the artist's skill. Most artists are right-handed and it might be easier for a right-hander to draw a profile to the left of the canvas (as it is easier to write from left to right).

Second, there could be bias in the positioning of the subject relative to the artist. A right-handed artist commonly holds the palette in his left hand so that his face, the palette and the subject's face are all in line and matching of colours from the palette is facilitated. The easel is usually to the right of him. Thus the artist is constrained to take up a position such that his left cheek faces the subject, and this position might be reciprocated by the subject to strike a balance.

Third, there might be bias in the social interaction between the subject and the artist. Independently of the constraints considered in the previous paragraph there might be a general (unexplained) tendency for any two people to feel more comfortable when they are positioned in such a way that their left cheeks face each other.

Table 2 Data in Terms of Body Visible

	Men				Women			
	Left	Right	% left	P	Left	Right	% left	P
Head only	142	166	46	NS	101	66	60	<0.01
Head and body	382	242	62	<0.001	265	111	70	<0.001

Fourth, there might be some visual preference for the left side of the face; perhaps it is considered more attractive than the right. Such a preference could possibly arise from some form of "exposure learning" whereby people come to prefer familiar visual stimuli[2]: Salk[3] claims that mothers tend to carry their babies on their left breast, so that a baby is exposed predominantly to the mother's left cheek. Alternatively the preference might reflect genuine differences between the left and right sides of the face resulting, perhaps, from fashion in make-up or coiffure (for example, parting of the hair over the left temple or placing beauty spots on the left cheek?).

Fifth, there may be superiority of the left visual half-field in facial recognition. Clinical evidence suggests that the right cerebral hemisphere (hence the left visual half-field) is superior to the right in recognizing pictures, especially pictures of faces[4]. A profile drawn to the left of the canvas might thus in a sense be more readily perceived.

Sixth, the way in which people turn their heads may be biased in favour of one direction. There might be a simple motor bias such that when the head is turned aside it is more likely to be turned to the subject's right than to his left. Informal observations do suggest that when people shake their heads (as if to say no) they tend to make the first move to the right.

But the data do not give much support to any of these explanations. The difference between men's and women's portraits cannot be accounted for by any of the hypotheses except maybe the fourth. The difference between "head only" and "head and body" portraits is contrary to the predictions of the first hypothesis and cannot convincingly be accounted for by any of the others.

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Perhaps the explanation in fact lies within the conventions of painting itself. Artists communicate more than mere physical likeness with their pictures: they attempt to convey character and status, and to do so they resort in part to arbitrary "signs". Baden-Powell instructed Boy Scouts to shake hands with their left hands in order to set themselves apart from other people: maybe when an artist paints his subject facing either to the left or to the right he is in some similar way imposing a structural classification which, as it were, puts the subject in his place. That such a system of signs should be unacknowledged either by the sender or receiver of the "message" would have parallels in other systems of human communication[5].

This last possibility, in some ways the most promising, is unfortunately the hardest to test experimentally. A semeiological analysis of the kind which has been successfully applied to symbolism in other forms of art[6] is unlikely to be fruitful when, as with the portraits of this study, too little is known *post hoc* of what message the artist intended to put over. An indirect test of the existence of a left/right sign system (which might also be used to test for left/right "preferences") would be to make mirror-image reproductions or unfamiliar portraits and to compare people's reactions to the original and its mirror-image with a technique such as the "semantic differential"[7]. A difference in people's attitudes to the same portrait reversed from left to right would argue strongly that the significant bias found in actual portraits is significant precisely because it does in fact signify.

Certainly one does not have to look far for anecdotal evidence that people have strong feelings about which side of their own face they would wish to put on show. The Royal Mint has recently revealed that when coin designs were first produced for King Edward VIII (late Duke of Windsor), he objected to those which showed the right side of his face. His misgivings, says the Royal Mint's librarian and curator, G. P. Dyer, arose from a firm conviction that the features on the left side of his face were superior to those of the right[8]. Superior is an interesting choice of word. It would appear from the original designs that the two sides of the King's face were, except for a left-side parting, almost perfect mirror images. Who knows but that it is a mark of a superior person to turn the left cheek to the world?

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Rifamycin Biosynthesis Studied with ^{13}C Enriched Precursors and Carbon Magnetic Resonance

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Studies using ^{13}C-enriched precursors in combination with carbon magnetic resonance spectroscopy have established the biosynthetic origin of most of the carbon atoms present in rifamycin S and suggest a common scheme for ansamycin biogenesis.

ALTHOUGH radiotracers, in particular ^{14}C, have been exceptionally useful in the analysis of biosynthetic pathways, they have one severe disadvantage: the necessity of carrying out extensive chemical degradations to establish the location of incorporated isotope. In the case of complex molecules one usually has to be satisfied with a partial analysis of isotope distribution.

The advent of ^{13}C as an alternative to ^{14}C in biosynthetic studies has led to the introduction of a technique which can completely establish the location of isotope within labelled metabolites. ^{13}C is a stable isotope present at 1.1% in natural organic molecules. Several ^{13}C-enriched compounds, suitable for use in biosynthetic studies, are commercially available.

Metabolites labelled with ^{13}C, obtained by adding enriched precursors to cultures, can be analysed by three techniques: mass spectrometry[1]; proton magnetic resonance spectroscopy[2–7] (PMR); and carbon magnetic resonance spectroscopy[8–17] (CMR). The most important of these approaches is the last one[18]. It is made possible by the fact that the nucleus of ^{13}C, unlike that of ^{14}C, has magnetic properties; also, recent advances in NMR instrumentation, in particular the introduction of the pulse and Fourier transform method, enable good natural abundance CMR spectra of most organic compounds to be obtained. Such spectra can be simplified by complete decoupling of ^{13}C from ^1H, all carbon atoms then appearing as single lines. The site of incorporation in a ^{13}C-labelled metabolite is revealed by an increase in peak height on its CMR spectrum compared with that obtained on the natural abundance spectrum.

Rifamycin Biogenesis

The rifamycins constitute a family of antibiotics first isolated in the Lepetit Research Laboratories in 1957 (ref. 19). Chemical[20] and X-ray crystallographic[21] studies showed that they consist of a naphthoquinone chromophore spanned by an aliphatic bridge, called the ansa chain (Fig. 1); they were the first examples of a new class of secondary metabolites for which Prelog has proposed the name ansamycins. Preliminary data (unpublished) of ^{14}C-precursor incorporation obtained in Prelog's and our laboratories led Corcoran[22] to propose a scheme for rifamycin biosynthesis in which the ansa chain was composed of five *propionate* and two *acetate* units. (Whenever a substrate is written in italics it indicates the chemical

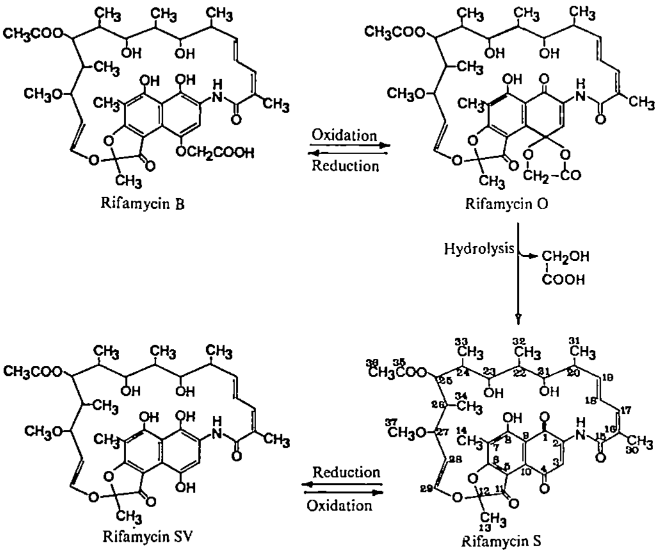


Fig. 1 Structural interrelationships of rifamycins B, O, S and SV.

entity which actually participates in the biological reaction.) The origin of the chromophore was not clear but appeared to contain only two *propionates*; the remainder was assumed to derive from *acetate*. Analysis of ^{14}C and ^{3}H-propionate incorporation has now been completed by Prelog (unpublished results) and only partially confirms Corcoran's proposal.

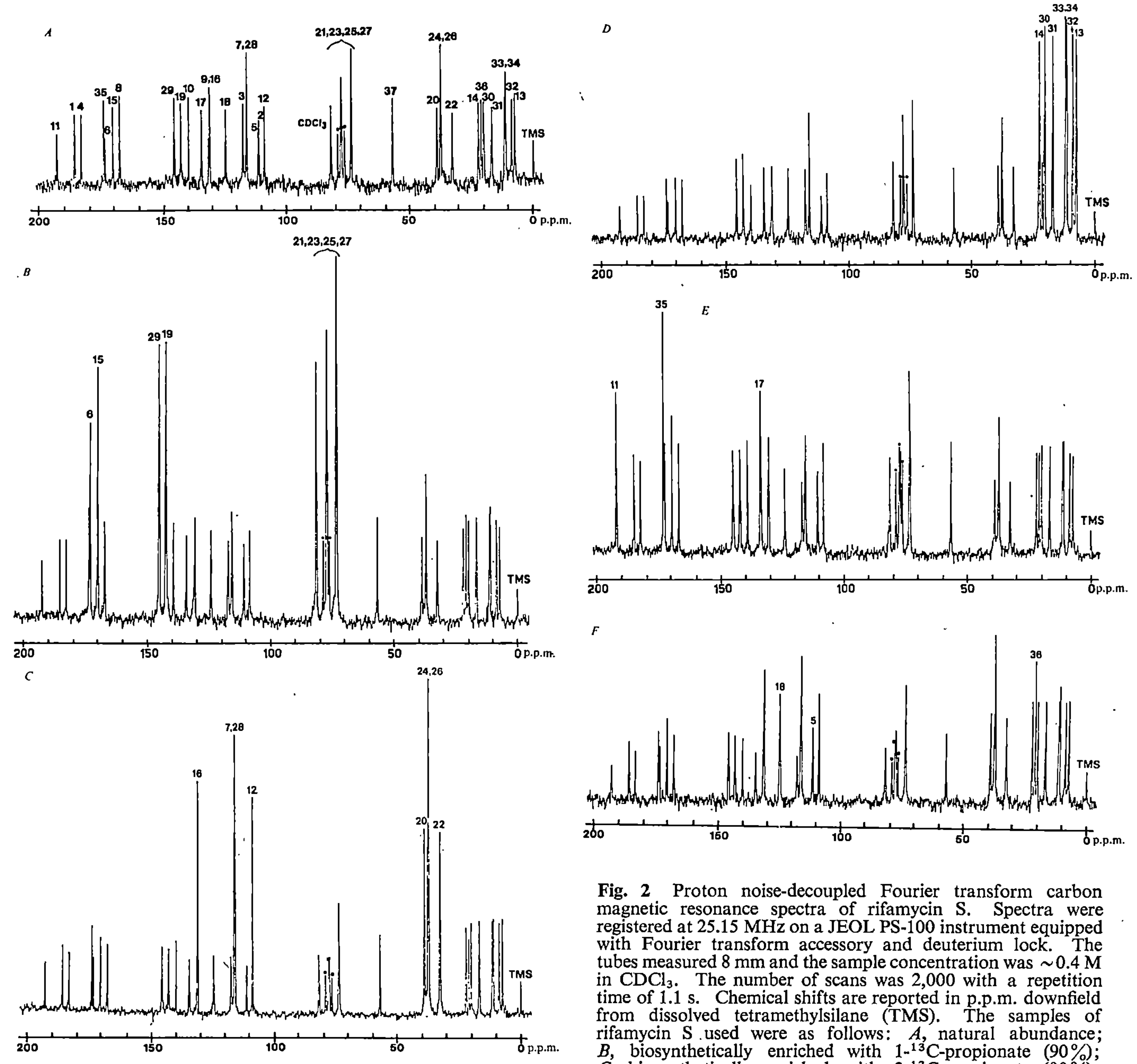


Fig. 2 Proton noise-decoupled Fourier transform carbon magnetic resonance spectra of rifamycin S. Spectra were registered at 25.15 MHz on a JEOL PS-100 instrument equipped with Fourier transform accessory and deuterium lock. The tubes measured 8 mm and the sample concentration was ~0.4 M in $CDCl_3$. The number of scans was 2,000 with a repetition time of 1.1 s. Chemical shifts are reported in p.p.m. downfield from dissolved tetramethylsilane (TMS). The samples of rifamycin S used were as follows: *A*, natural abundance; *B*, biosynthetically enriched with 1-^{13}C-propionate (90%); *C*, biosynthetically enriched with 2-^{13}C-propionate (90%); *D*, biosynthetically enriched with 3-^{13}C-propionate (64%); *E*, biosynthetically enriched with 1-^{13}C-acetate (90%); *F*, biosynthetically enriched with 2-^{13}C-acetate (90%). Precursors enriched with ^{13}C were all purchased from Merck Sharp and Dohme Ltd, Montreal, Canada.

Although rifamycin B is the normal fermentation product, biosynthetic studies[23] have centred on the simpler rifamycin S, which is the precursor of this family of antibiotics[24,25] and which had previously been obtained from rifamycin B by successive oxidation and hydrolysis, with the intermediate formation of rifamycin O (Fig. 1). Simple reduction of rifamycin S yields rifamycin SV.

Incorporation of ^{13}C-Acetate and ^{13}C-Propionate

In the experiments described here, samples of rifamycin B enriched with ^{13}C were prepared by addition of ^{13}C-propionate and ^{13}C-acetate to fermentations of *Nocardia mediterranei*, carried out according to known procedures[26]. Concentrations of more than 200 μg ml^{-1} of acetate and propionate are toxic, and it proved necessary to pulse-feed precursors twice a day over 96 h to obtain a satisfactory incorporation. At the end of the fermentation ^{13}C-enriched rifamycin B was isolated, converted to rifamycin S and then examined by CMR spectroscopy.

The natural abundance proton-decoupled CMR spectrum of rifamycin S is shown in Fig. 2*A*; the attributions given will be the subject of a separate publication[34].

Assignments within the two groups of carbon atoms C-20, C-22, C-24, C-26 and C-31, C-32, C-33, C-34 are only tentative and those within a third group have not been established. Conclusive assignments within these three groups must await the appropriate selective proton decoupling experiments, but fortunately none of these uncertainties affects the biosynthetic argument as the members within each group have the same metabolic origin.

The CMR spectra of the five samples of labelled rifamycin S obtained from the different ^{13}C-enriched precursors are shown in Fig. 2*B–F*. Peak heights on spectra of the propionate-labelled samples show that: carbons 6, 15, 19, 21, 23, 25, 27 and 29 derive from C-1 of propionate; carbons 7, 12, 16, 20, 22, 24, 26 and 28 derive from C-2 of propionate; carbons 13, 14, 30, 31, 32, 33 and 34 derive from C-3 of propionate. Thus, there are eight *propionate* units in rifamycin S, one of which has lost its methyl (from C-28), during biosynthesis.

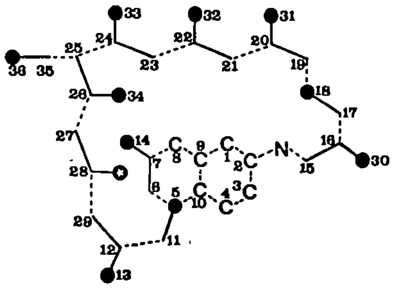


Fig. 3 Alignment of *acetate* and *propionate* residues in rifamycin S. Filled circles represent the methyl groups of the propionate (●—/) and acetate (●——) residues. Bonds joining the carbon atoms of subunits are shown as solid lines and bonds joining the subunits as broken lines. The "odd" propionate methyl on C-28 (✪) is lost during biosynthesis.

Interpretation of the spectra obtained for rifamycin S biosynthetically enriched with ^{13}C-acetate is complicated by the concurrent, indirect incorporation of the precursor through *propionate*. This is particularly evident in the case of 2-^{13}C-acetate where many carbons are enriched. Comparison with the ^{13}C-propionate spectra reveals that all carbons enriched by 2-^{13}C and 3-^{13}C-propionate are also enriched by 2-^{13}C-acetate. This is in agreement with the expected metabolic conversion of acetate to *propionate* during the Krebs cycle. In addition to this indirect incorporation three carbons (C-35, C-17 and C-11) are enriched by 1-^{13}C-acetate and three (C-36, C-18 and C-5) by 2-^{13}C-acetate.

Although quantification of data obtained on proton-decoupled spectra is made difficult by the nuclear Overhäuser effect[27], an approximation of the degree of enrichment in a particular carbon atom can be estimated from peak heights on enriched and natural abundance spectra. When this is done for the six acetate-derived carbons, those of the acetoxyl group (C-36, C-35) appear more enriched than the other two units (C-18, C-17 in the ansa chain, and C-5, C-11 in the chromophore). This is shown in Table 1, where the data for a representative *propionate* unit are included for comparison. These data are in agreement with a different metabolic origin for the acetoxyl and the other two units. Secondary metabolites containing *acetate* and *propionate* units are probably synthesized in the same way as fatty acids; that is acetyl CoA and propionyl CoA function as initiators whereas malonyl CoA and methyl malonyl CoA participate in chain extension[28]. The two *acetate* units C-5, C-11 and C-18, C-17 are clearly in chain extension situations and should derive from malonyl CoA, giving a lower incorporation at these two positions in comparison with the acetoxyl group which must derive more directly from acetate (by way of acetyl CoA?). Furthermore, incorporation of ^{13}C-acetate through *propionate* is of the same order of magnitude as that through malonyl CoA.

Table 1 Estimated Incorporation of ^{13}C-Acetate

Carbon atoms	Enrichment factor* 2-^{13}C-Acetate	1-^{13}C-Acetate
C-5	1.9	1.4
C-11	0.8	2.4
C-18	2.0	1.0
C-17	0.9	2.0
C-36	2.6	1.2
C-35	1.1	3.0
C-30	1.9	1.4
C-16	2.0	1.1
C-15	1.5	1.8

* Peak heights $\frac{\text{enriched sample}}{\text{natural abundance}}$ from spectra run under essentially identical instrumental conditions and corrected for difference in concentration.

No such differences in enrichment are evident if similar calculations are performed for the samples biosynthetically enriched with ^{13}C-propionate. When the origin of the twenty-nine carbons deriving from propionate and acetate is summarized (Fig. 3), it can be seen that none of the *propionate* units is in a chain initiating situation. Therefore we assume that all propionate incorporation occurs through methyl malonyl CoA; in fact ^{14}C-methyl-malonate gives essentially the same ratio of labelling for ansa and chromophore as ^{14}C-propionate (unpublished results).

Direction of Chain Growth

Figure 3 shows that the ansa chain can be made by a regular, clockwise condensation of *propionate* and *acetate* units; this is specified by the orientation of the *acetate* unit whose methyl group gives rise to C-18. In this respect the attribution of C-17 and C-19 was crucial, as an interchange would indicate the reverse direction of chain growth during biosynthesis. It was therefore unambiguously established on the basis of specific proton decoupling experiments.

The ansa chain is interrupted between C-12 and C-29 by an ether linkage. The unusual labelling pattern and the fact that the *propionate* and *acetate* units in the ansa chain and chromophore are equally labelled suggest that C-13, C-12 and C-29 originate from a single propionate unit subsequently split by the introduction of an oxygen atom. In this way it is possible to construct rifamycin S from a single polyketide chain, initiated by a seven carbon aromatic moiety.

Origin of Remaining Carbon Atoms

Earlier work with ^{14}C showed that the methoxyl group C-37 comes from methionine along the normal, folate-mediated pathway (unpublished results). Thus we are left with seven carbons and a nitrogen of unknown origin. Our results disagree with the recent suggestions[29] that the naphthoquinone moiety arises by condensation of mevalonate with a phenolic compound and that it is synthesized separately from the ansa chain. Surprisingly, the acetate-plus polymalonate pathway, a common means of aromatic biosynthesis in the Actinomycetales, is not responsible for production of rifamycin chromophore as only one *acetate* unit is present. We have been unable to obtain a specific incorporation of other known ^{14}C-labelled precursors of aromatic rings, such as shikimate, into this part of the molecule.

A Common Ansamycin Progenitor?

Two other families of ansamycins with naphthalenic chromophores are known: tolypomycins[30] and streptovaricins[31]. Many of the differences between and within these groups of antibiotics can be recognized as variations commonly seen among metabolites deriving from the same progenitor. But certain differences seemed indicative of a different sequence of building blocks in the original chain; thus C-28 and C-29 in the rifamycins and tolypomycins appeared to derive from *acetate* whereas the analogous carbons in the streptovaricins clearly come from *propionate*. Our results show that all three types of ansamycin can derive from the same fundamental molecule.

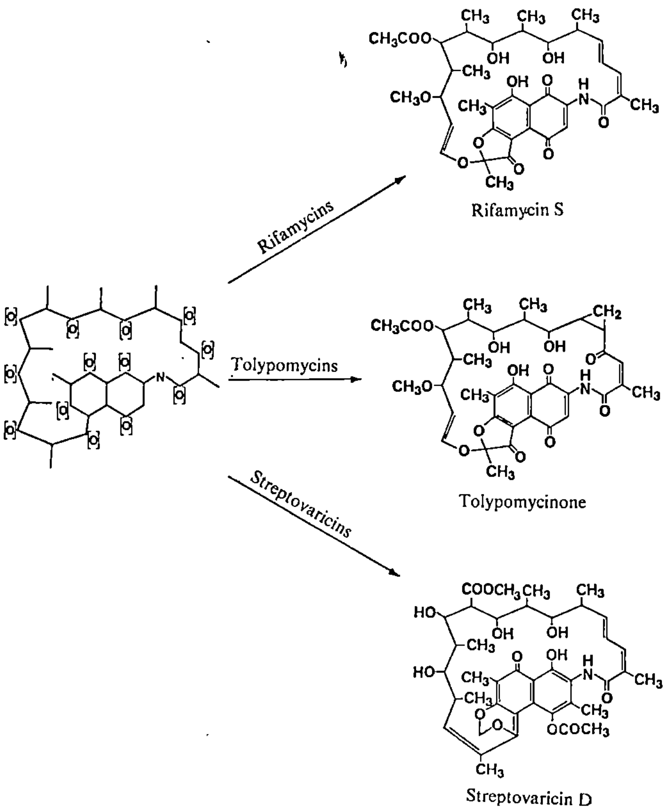
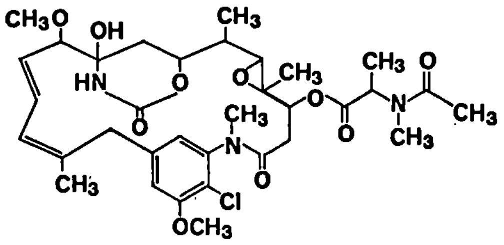


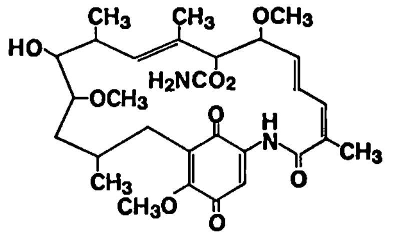
Fig. 4 Carbon skeleton of hypothetical common progenitor and its relationship to the naphthalenic ansamycins. Oxygen functions are bracketed as a result of uncertainties in the timing of their introduction or modification.

The carbon skeleton of the hypothetical progenitor and its relationship to the naphthalenic ansamycins is shown in Fig. 4. While the streptovaricins maintain all the fundamental features of this precursor, the tolypomycins and rifamycins would be formed by introduction of an oxygen between C-12 and C-29, and loss of the methyl from C-28 (the numbers refer to those given for rifamycin S in Fig. 1). Enzymes involved in the synthesis of secondary metabolites quite often lack the absolute specificity characteristic of primary metabolism, making it difficult to establish an unambiguous sequence for the biosynthetic reactions. Thus, some of the subordinate modifications, such as introduction of double bonds or methylation at C-3, may occur before or after completion of the carbon skeleton.

Two ansamycins characterized by benzenic chromophores have recently been discovered, geldanamycin[32] and maytansine[33] (see Fig. 5), the latter, in contrast to all other ansamycins, is a plant product. In both cases, it seems that a similar scheme of biogenesis operates and that the precursor of the aromatic part is the same as that for the other ansamycins. It is tempting to suggest that absence of the appropriate oxygen function "ortho" to chain attachment has prevented closure of the second ring and hence formation of a naphthalenic chromophore.



Maytansine



Geldanamycin

Fig. 5 Structures of the benzenic ansamycins, geldanamycin and maytansine.

Advantages and Limitations of the Technique

The results obtained with rifamycin S illustrate the usefulness of ^{13}C-enriched precursors in conjunction with CMR as a tool for studying the biosynthesis of complex molecules.

Successful application of this approach depended largely on two favourable factors: first, a range of rifamycin derivatives was already available which permitted interpretation of the natural abundance CMR spectrum; second, a high level of precursor incorporation could be obtained. The use of ^{13}C can avoid recourse to the chemical degradations associated with radiotracer techniques, but interpretation of the CMR spectrum may itself require a considerable chemical effort. Although very high levels of incorporation have occasionally been obtained, the extent of enrichment required with ^{13}C will probably limit a generalized application of this approach, and it must be remembered that the metabolic pattern itself may be altered by the substrate amounts of precursor necessary to obtain such an enrichment.

The main outcome of our results is that the ansamycins, which were previously considered to be a group of natural products on purely structural grounds, can now be considered a family from the biosynthetic point of view, deriving from a polyketide chain initiated by the same aromatic moiety. It is doubtful that the same conclusions could have been reached with the conventional radiotracer approach.

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Continental Drift and Reserves of Oil and Natural Gas

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The necessary physiographic location of deltaic sources deposited during the past 200 million years requires that continental movements be taken into account.

Any new concept which is likely to assist in the location and assessment of the remaining reserves of oil and hydrocarbon gas is of obvious importance so it is opportune to use reviews[1,2] of the conditions of formation of the principal hydrocarbon sources and their subsequent migration and preservation in reservoirs to see how these are related to movements of the Earth's surface.

Deposition of Organic Substances

The ability of hydrocarbons to migrate[3] and their complex chemical nature complicates matters but it is generally agreed that they are organically derived[4,5], the oil coming chiefly from animal proteins and the gas including some vegetal content[1]. Sources some 10^7 yr old are almost entirely deltaic and occur independently of latitude, the organic materials being trapped within the rapidly accumulating sediments. The rich terrestrial vegetation of tropical areas means that there may be some latitudinal control on the percentage of gas to oil, but this is unlikely to be as significant as later factors. The location of deltas today is evident but somewhat older deltas exist which receive little or no drainage today and are therefore harder to distinguish physiographically as continental movements have separated the headwaters from deltas which now lie isolated and unexploited on other continents. The formation of mountain systems, along subduction zones or by continental collisions, also drastically changes the drainage pattern so that, for example, dispersed oil fields of the Andes may once have formed part of the proto-Amazon delta[6] when the Amazon drained from Africa. Deltaic areas older than 200 m.y., which covers the "Wegenerian" phase of continental drift, are almost impossible to locate physiographically but are of less economic importance because most productive deltaic areas are less than 200 m.y. old and geological identification is possible, irrespective of age.

Shales are generally considered to have the highest initial organic content, followed by carbonates and finally sandstones—although sandstones are the principal rock type for major reservoirs[1] and can be a significant contributor to their own reservoirs. But the productivity of source rocks depends on the ease of migration of the hydrocarbons so that the most productive source rocks are the massive carbonates, although the productivity of all source rocks is increased by heating. Deep oceanic sediments accumulate slowly and are unable to trap significant protein matter before oxidation so that deep oceans are not potential oil or gas sources, except where shallower sediments have slumped into them, but much of the hydrocarbon is also likely to be lost at this time.

Because more than 90% of known oil and gas fields[1,7–9] are associated with evaporite deposits the conditions for evaporite accumulation are relevant to the source, migration and preservation by hydrocarbons. Most marine evaporites are formed of anhydrite, with some gypsum, and contain only small amounts of the more soluble halite. Even when halite forms a large part of the deposit, it only represents temporary conditions as it was laid down some 10^2 to 10^3 times faster than anhydrite[10]. Significant deposits cannot therefore have formed by prolonged, simple evaporation of seawater because halite would always predominate. Two depositional environments have been postulated to account for these features—the silled basin and the sabkha. The former[10–12] consists of a pool of water into which only surface waters can flow. As these surface waters evaporate, saline brines form and, being denser, sink to the bottom where the least soluble salts are eventually precipitated; but the salinity can only rarely increase to the concentration appropriate to halite deposition. "Sabkha" regions[13,14] form from the occasional flooding of supratidal flats which create extensive areas of shallow marine waters. As these waters evaporate and retreat, the least soluble salts are left behind. These deposits are greatest where the flats are bordered by lagoons in which the salinity is already higher than normal seawater. Such extensive flat regions would be more common when orogeny was less active than during the past 20 m.y. and when sealevel was more constant than during the eustatic changes of the most recent Ice Age.

In both environments the depositional area must sink relative to the barrier and sealevel to account for the observed thicknesses of evaporites. Rapid subsidence in areas of low evaporation would cause persistent flooding and the solution of previous salts, as would also happen with rare rainstorms in areas of low evaporation and slow subsidence. So although both types of environment could exist temporarily at any latitude, thick deposits could only form in regions with a very high rate of evaporation, that is where hot, dry conditions are accompanied by strong diurnal on-shore and off-shore winds. Cyclothems and structures in associated sediments indicate intermittent subsidence so that the evaporation rates would have to match the maximum rate of subsidence. Such prolonged subsidence implies movements of the lower crust and upper mantle, as occurs in response to isostatic adjustments, for example during continental separation[15]. For continued deposition, the barriers of a silled basin and the shoreline of a sabkha must maintain their position relative to the subsiding depositional area. A fault controlled structure, where very localized tectonic movements happen to cancel out regional movements, could create this situation, but it is more likely that such persistent barriers or shorelines would result from sedimentary or biological processes. Sandbars could be maintained relative to sealevel if marine currents and a sediment supply were unaffected by the subsidence, but the most persistent type of barrier/shoreline would be formed by shallow reef structures. Reefs in low latitudes would easily maintain their position relative to an intermittently deepening sea as their growth is optimized in shallow, warm, clear waters. These low latitude conditions, necessary for optimum evaporite formation, are bordered by those for the optimum production and preservation of hydrocarbon source materials as the concentration of nutrients in warm, saline waters results in rich organic blooms of microorganisms, the salinity restricts the entrance of larger organisms which could feed on them and the nearby anaerobic bottom conditions are ideal for the preservation of the organic debris[16–18].

Migration

The gradual freeing of hydrocarbons from the original organic matter occurs continuously but is accelerated by small temperature rises[19,20] to which plate tectonic theories and continental drift have varying degrees of significance. Tectonic heating during collision between two continental blocks is likely to be locally severe and drive off all hydrocarbons, but the less disturbed areas and margins of collision zones may be areas of significant oil and gas migration. Such areas can be located from palaeomagnetic measurements of the angle and rate of collision but the shape of the original edges is also critical and this can only be detected by detailed geological and geophysical studies which, among other things, yield the information of sources, migration and reservoirs. Similarly, in subduction zones there is higher heat flow and igneous activity in a region up to 1,000 km wide, but the distribution of heat is not regular and the local controlling factors are only just becoming clear[21] and the estimation of heat flow in different areas, now and in the past, should become more precise as such interacting factors are considered further.

The igneous activity and high heat flow along spreading oceanic ridges are not of any importance within the oceans, where there are no source rocks, but become highly significant when these ridges intersect, or have intersected, continental plates. Local destruction of reserves is likely where significant igneous activity was involved, such as at previous plume centres, but these will also be rimmed by high gas pressures. In general, the high heat flow of the ridges will have been a major cause of migration as the hydrocarbons would move slowly from deep to shallow levels as the regional temperature increased. Thus the numerous, highly productive gas fields of north-west Siberia occur over the continental extension of the Arctic ridge; similarly the productive oil fields around Los Angeles[22] are where the East Pacific Rise underlies continental rocks. So the identification of present and past intersecting regions is likely to be of great importance.

As the Atlantic and Indian Oceans originated from the break up of the Gondwanan and Laurasian continents, the fractured edges must have been subjected to oceanic ridge heating at their inception. This heating would coincide with the accumulation and migration of organic materials in these areas as the persistent subsidence afforded troughs into which organic material could be trapped and also allowed a thick accumulation of sediments to develop in them, giving rise to burial heating of older sediments. In general, direct heat flow is likely to have been most significant along the margin[23] and burial heating would be dominant within a few hundred kilometres of the margin. This subsidence was usually along pre-existing fractures where these paralleled the opening margins and the deepest subsidence took place on the continental side of fault blocks as they respond to upper mantle and lower crustal flow. Seafloor spreading also seems to be the cause of the formation[24] of marginal basins, such as the Japanese Sea, which also contain shallow sedimentary rocks. Thus the availability of source rocks and heating by burial and elevated heat flow implies that these regions, particularly in the West and Southwest Pacific, are likely to be areas of high hydrocarbon potential.

On the plate tectonic model igneous activity and high heat flow are absent away from plate margins, with the minor exception of kimberlite-type intrusions[21]. Deep burial heating can still occur away from plate margins as a result of upper mantle/lower crustal movements, but significant heating in such areas presumably arises mainly from exothermic chemical and biochemical reactions which may accompany diagenesis and subsequent compaction. Most of these reactions are independent of plate tectonic concepts, but some important reactions, such as between organic materials and anhydrite[13], will occur chiefly in sediments originally deposited at low latitudes.

The final composition of the reserves reflects the nature of the source materials, the temperature and organic activity in the source, during migration and after preservation in reservoirs, and the distance between source and the reservoir. So fatty, heavy grade oils are particularly associated with deltaic conditions[25] where migration has been restricted and the original organic material is often derived from brackish environments. Deltas also tend to have substantial gas associated with their high vegetal content. The variations of sulphur content within oils, or nitrogen within gas, are significant to extraction and refining techniques and prediction of the nature of the rocks through which the hydrocarbons have passed by determining their palaeolatitudinal history will lead to a closer understanding of natural refining processes and therefore to a prediction of the quality of hydrocarbon reserves. Sulphur content[26,27], for example, seems to correlate broadly with the presence of evaporites in the source rocks, but the final composition is probably determined largely by bacterial activity, particularly within the reservoir rocks.

Reservoirs

Various structures may trap oil and gas[5] and plate tectonic activity is involved in each, although some links are tenuous. Stratigraphic traps, for example, are related to the detailed local palaeogeography, but the number of traps in any one region is controlled by broader considerations. For example, structural traps, such as anticlines and domes, are directly related to tectonic processes along subduction and continental collision zones. Thus the gas fields marginal to the Pyrenees owe their origin to the relative movements between Iberia and France and the Argentine fields probably formed in response to movements of the Patagonian arc[6].

Evaporites are again significant because they lubricate tectonic movements, and afford an impervious seal for trapped hydrocarbons. Their ability to flow when the overburden exceeds some 600 m (ref. 28) means that they can create their own structural traps. The role of reefs and sand bars in evaporite formation also means that these offer potential reservoirs close to hydrocarbon sources. The margins of separating continents also provide structures, accompanying the faulting and folding movements, which may act as reservoirs.

Implications for Fuel Supplies

Concepts of continental drift and plate tectonics have a much more fundamental part to play in the evaluation of potential oil and gas reservoirs than in the mere matching of, for example, oil traps bordering the Atlantic. Physiographical location of deltaic sources deposited during the past 200 m.y. can be undertaken effectively only when continental movements are taken into account and similarly the structural evolution of an area should be related to the different distribution, movements, collisions and separations of the continents. The most striking feature is that palaeoclimatic consideration plays a vital part in the origin, migration and accumulation of hydrocarbons. Palaeomagnetic techniques, which offer a precise, economic measure of palaeolatitude, are therefore likely to be of increasing significance.

As technology advances, it should be possible to extract oil and gas from some of the extensive tar shales as well as from inhospitable yet potentially productive areas such as the Labrador Sea, Newfoundland Shelf, northern Greenland and the Canadian Arctic Islands. Nonetheless, the fact that low palaeolatitudes are so significant suggests that the southern continents which, as Gondwanaland, have mostly lain at high to intermediate palaeolatitudes during the past 400 m.y., are unlikely to be as productive as the northern hemisphere—although the size of Gondwanaland implies that some areas must have been at low latitudes for appreciable periods of time. So something like two-thirds of the world's total reserves are likely to be in the present northern hemisphere, which are already the best known areas and the regions of highest exploitation. Major reserves which are largely undetected or unexploited must still exist, for instance, off north-western Australia, the south-west and western Pacific, as well as in uptapped Mesozoic-Cainozoic deltas. It is also clear that the deep oceanic parts of the world are not likely to contribute to future needs for organic fuels. It should now be possible to make a realistic estimate of the world's hydrocarbon potential and thereby evaluate the optimum development of these resources for the benefit of mankind as a whole.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Origin of Broad Emission Lines from Quasistellar Objects

THE optical spectra of QSOs exhibit very broad high-excitation emission lines. The broadest of these in the visible region for typical redshifts are the resonance lines Lyα λ1216, CIV λ1550 and MgII λ2798 (ref. 1). Previous suggestions for the origin of the breadth of these lines include electron scattering and Doppler shifts due to turbulence or other mass motions in clouds of gas.

We have recently proposed a radially-streaming-proton model that gives an excellent fit to profiles of the broad Hα emission lines from three different Seyfert galaxy nuclei (ref. 2 and our unpublished work). We suggest here that a similar idea will also explain the observed width of QSO emission lines. In the model, suprathermal protons slow down as they stream radially through a hydrogen gas that is at rest with respect to their source at the centre. Hydrogen emission from these protons occurs when they have slowed to speeds less than about 5,000 km s^{-1} (about twice the speed of an electron in the first Bohr orbit), and are able to carry an electron for an appreciable fraction of the time. Thus the hydrogen emission profiles are predicted to all have overall Doppler widths at near zero intensity of about 10,000 km s^{-1}.

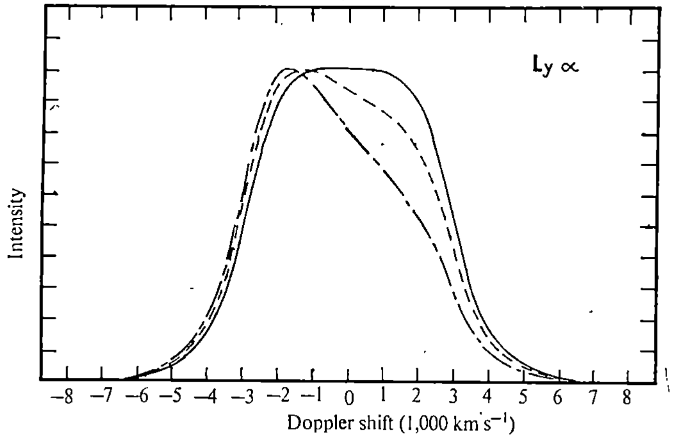

Fig. 1 Profile of Lyman alpha resulting from a radially-streaming-proton model for three different values of the transparency of the emitting region. The parameter Λ is the ratio of the photon mean free path to the radius of the emitting sphere: ——, $\Lambda=\infty$; - - -, $\Lambda=5.0$; - — -, $\Lambda=2.0$.

In Fig. 1 we display three profiles for Lyα computed in such a model using our (unpublished) atomic cross sections. The profiles differ only in the assumed transparency of the region where the emission originates, which is the only parameter in the model that changes the profile. The overall width is determined by atomic constants; the width of the profile, measured between the two inflexion points at complete transparency, amounts to $\Delta\lambda/\lambda$ equal to 3.3 times the fine structure constant. The total emission in Lyα from the streamers is computed to vary from about 600 photons per proton when the gas is neutral, to about 6 photons per proton at 90% ionization for the gas.

We have made preliminary calculations of profiles for CIV λ1550, again assuming that the carbon nuclei are decelerating as they stream radially through a hydrogen gas. The necessary atomic cross sections are known with less certainty than for the Lyα case so the width is less well defined. It is clear, however, that the shape of the CIV resonance line should resemble the Lyα profile, especially in the wings, but be broader by a factor of approximately the square root of the ratio of the ionization potentials of CIV and hydrogen (about 2.2). Because it is primarily the speed of the outermost electron of the ion that determines the behaviour of the atomic cross sections for charge transfer and ionization, the widths of profiles of resonance lines of other ions can be predicted by the same scaling procedure.

There seem to be very few published high-resolution profiles of the broad emission lines from quasistellar objects, but those we have seen[3] seem to agree with what we have calculated. Low resolution observations have been made on several QSOs by Oke[4,5]. A detailed comparison of calculated and observed profiles is not meaningful at this resolution (about 50 to 100 Å), but it is interesting that the hydrogen lines are typically quite broad with a full-width at zero intensity on the order of 10,000 km s^{-1}, and the resonance line of CIV seems to be broader still. The data for 3C273 are consistent with half-half widths of the Balmer lines of about 2,500 km s^{-1}, which is what one expects from our model. What is needed for a good test is a determination of these profiles with a resolution of a few angstroms.

Because of apparent association of QSOs of different redshifts, the failure of QSOs to conform to the same Hubble law as known galaxies and the different redshifts of emission and absorption lines in the same QSO, it has been suggested that new physical laws may be required to describe them (ref. 6 and G. Burbidge, unpublished). If the profiles we have computed agree with QSO emission profiles as well as they do with the hydrogen emission profiles of Seyfert galaxies, then it will be strong evidence that at least the fine structure constant must have the same value in a QSO as it does in the Solar System.

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Palaeomagnetic Evidence for Rotation of Sardinia during the Early Miocene

GEOLOGICAL and palaeomagnetic evidence indicates that after the Permian the Corso-Sardinian plate rotated counterclockwise with respect to stable (extra-Alpine) Europe[1–6]. Palaeomagnetic directions from Late Oligocene/Early Miocene rocks can be interpreted as indications for a Late Tertiary rotation[5,7]. Here we present additional palaeomagnetic data from volcanic rocks and tuffites of Tertiary age, collected near the village of Castelsardo on the northern coast of Sardinia (Fig. 1).

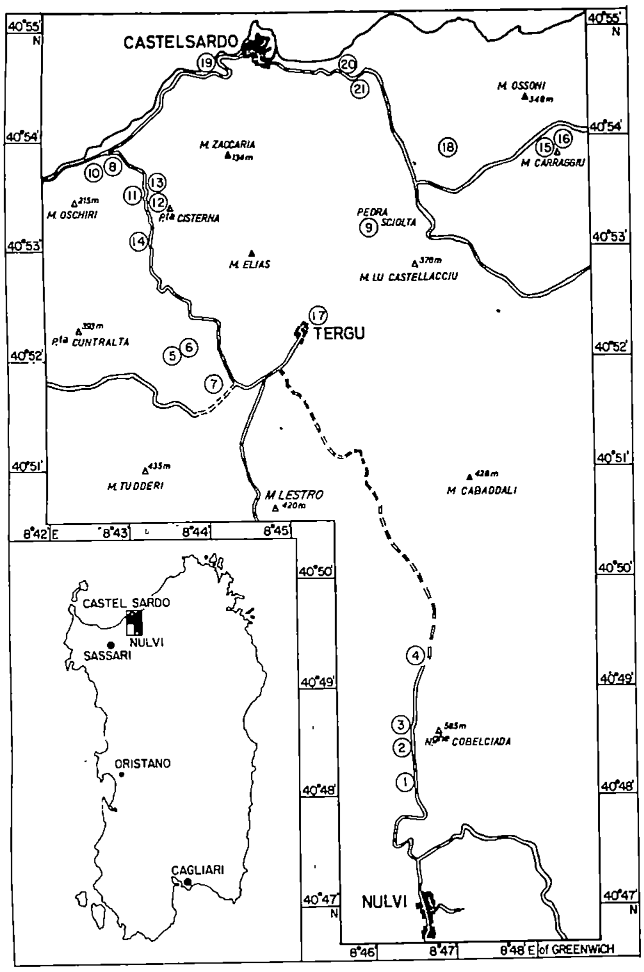

Fig. 1 Map of the Castelsardo area with sampling sites indicated.

The volcanic rocks of northern Sardinia consist of andesites, trachytes and liparites. Tuffs, tuffites and sediments are intercalated. The exposures are discontinuous, the sediments show rapid facies transitions, and block faulting hampers detailed correlation of the volcanic rocks. Near Castelsardo, however, it is possible to map in detail a number of stratigraphical units, from which oriented hand samples were collected. The geological relationships of these units are depicted schematically in Fig. 2. The two principal andesite bodies in the area (indicated as α on the geological map of Moretti *et al.*[8]) underlie all other sampled units, the oldest of which are Aquitanian. Comparison with other volcanic units outside the Castelsardo area suggests that the andesites are not older than Middle Oligocene[9]. Their age must be similar to that of the Alghero trachyandesites; a contemporaneity supported by similar palaeomagnetic directions.

The overlying lacustrine sediments, tuffs and tuffites are dated as Aquitanian/Langhian on the basis of contained fossils (refs. 10, 11 and M. L. Colalongo, personal communication). Intercalated in the sediments and tuffites (M1t) are two flows of trachyte (M1T). Samples from two sites within M1t and from seven sites within M1T yielded similar palaeomagnetic results. Small local andesite plugs and intercalations also occur, but they have not been sampled extensively because of uncertain stratigraphical relationships.

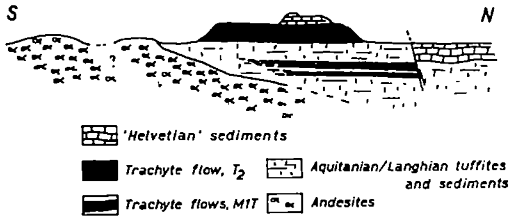

Fig. 2 Schematic stratigraphical section of the sedimentary and volcanic rocks which were sampled for this study.

There is a thick trachyte flow capping the sequence of M1t sediments, tuffs and M1T flows. Southeast of Castelsardo this flow is usually denoted as T_2, southwest of Castelsardo $T_2\delta$ and T_3 are also used. Sampling was restricted to the exposures denoted as T_2, because the geological correlation between the T_2 exposures is unambiguous and because its age is better established: for instance 2 km southeast of Castelsardo, the flow is overlain by marine sediments of Serravallian (Helvetian auct.) age[11].

The natural remanent magnetizations of ninety-five hand samples from twenty-one sites have been studied in the

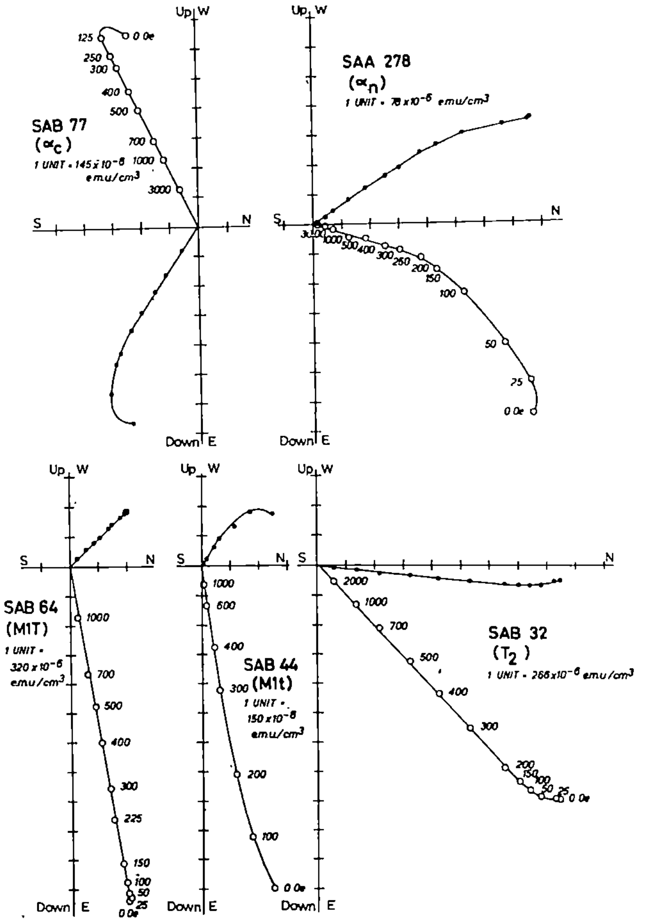

Fig. 3 Demagnetization diagrams obtained from samples from the five stratigraphical units investigated. Plotted points represent the end points of the magnetization vector—in orthogonal projection—during progressive AF demagnetization. Full symbols represent projections on the horizontal plane; open symbols represent projections on the N–S vertical plane.

Palaeomagnetic Laboratory of the State University of Utrecht. The intensities of the magnetizations were of the order of 200×10^{-6} to $9{,}000 \times 10^{-6}$ emu cm^{-3}; the Q-values varied between 0.4 and 30, but were generally higher than 1.0. Stepwise demagnetization in alternating fields of up to 3,000 Oersted generally revealed that secondary magnetizations play no important role and that they can be easily removed in the lower alternating fields (Fig. 3). Fields of 3,000 Oersted usually also completely eliminated the characteristic magnetizations. The direction of the characteristic magnetization was determined from the final straight trajectory of the demagnetization diagrams (for example, the straight paths between 300 and 1,000 Oersted in Fig. 3). The "within-site" scatter of the directions of magnetization was very small and generally decreased even more on demagnetization.

In Fig. 4 we plot the mean site directions of these stratigraphical units (with respect to the original horizontal) together with those previously published for Tertiary rocks from Sardinia. Both the inclination and the declination values show a large variability. The variability in declinations is remarkable because here there seems to be a consistent trend: the oldest rocks show the most westerly declinations (Fig. 4*a*), younger rocks a NNW declination and the youngest rocks (flow T_2 and the two Plio-Pleistocene basaltic formations β_1 and β_2) possess a direction close to due north (Fig. 4*b*).

This change in declination could be the result of a long lasting Late Oligocene–Early Miocene excursion of the geomagnetic dipole field, but such an event has not been demonstrated for this period. We conclude, therefore, that it is more likely that the change in declination represents a

Table 1 Summary of the Tertiary Palaeomagnetic Data from Sardinia

Age	Formation	Map symbol[8]	Site No. Fig. 1	Decl. Incl. per site *	Mean direction of magnetization					Pole position and (dp, dm)
					N (n) †	K	α_{95}	Decl.	Incl.	
Post-Tortonian, mainly Pliocene and Quaternary	Basalts near Logudoro[7]	β_1		14.5+56.5 353.5+52.5 347.5+49.5	3 (8)	80	14°	357.5	+53.5	83 N, 154 W (13.5; 19.5)
	Basalts near Logudoro[7]	β_2		360 +46.5 355.5+46.5 351 +41	3 (9)	321	7°	355.5	+44.5	75 N, 155.5 W (5.5; 8.5)
	Basalts near Orosei[13]	?		181 −62 199 −50 174 −51 180 −55 181 −55	5 (34)	120	7°	183	−55	84.5 N, 162.5 E (7; 10)
	Basalts from Campidano[13]	?		183 −51	1 (9)	–	–	183	−51	81.5 N, 171 E (9; 13.5)
	Basalts near Campeda[13]	$\beta_1+\beta_2$		342 +50	1 (5)	–	–	342	+50	72.5 N, 108.5 W (9; 13.5)
Middle/Late Miocene	Castelsardo trachyte (our work)	T_2	17 18 19 20 21	359.5+51 359 +46 6.5+52 5.5+46.5 7.5+62.5	5 (25)	131	6.5°	3.5	+51.5	81 N, 170 E (6; 9)
Early Miocene	Aquitanian/Langhian tuffites of Castelsardo (our work)	M1t	8 9	334 +64 321.5+73.5	2 (9)	120	23°	329	+69	66 N, 42 W (33; 39)
	Aquitanian/Langhian trachyte flows near Castelsardo (our work)	M1t	10 11 12 13 14 15 16	324 +72.5 327.5+69 287 +62 301 +56 272 +51.5 295.5+55.5 305.5+56	7 (32)	46	9°	298.5	+61.5	45 N, 57.5 W (10.5; 14)
Oligocene–Early Miocene	Andesite body, south of Castelsardo (between Nulvi and Tergu (our work)	α_n	1 2 3 4	325.5+11.5 318.5+12.5 325.5+ 5 326 +14	4 (17)	234	6°	324	+11	42.5 N, 119 W (3; 6)
	Andesite of Contralta near Castelsardo (our work)	α_c	5 6 7	130.5−65 110 −62.5 119 −48.5	3 (11)	64	15.5°	119.5	−59	45 N, 62 W (17.5; 23)
	Alghero trachyandesites[5]	T_1		148 −47 317 +43 324.5+42.5 341 +16 153 −40 162.5−43 146 −28 138.5−43 284 +54.5 283 +49	10 (59)	20	11°	322	+42	54 N, 95 W (12.5; 17)
	Pre-Helvetian volcanics near Logudoro[7]	α (α_1 ?) T_1 α (α_2 ?) T_2 T_3 T_3' (2) α_3		344 +66.5 120.5−35.5 307 +49 341.5+29.5 345 +24.5 337 +64.5 169 −25.5	7 (28)	12	18°	332.5	+43.5	62.5 N, 106.5 W (14; 22.5)

Geographical data according to the 1 : 100,000 sheets of the Geological Map of Italy.

* After demagnetization (500–3,000 Oersted) and after correction for the dip of the beds.

† N, Number of sites or flows, used in the statistical analysis; n, the number of samples investigated.

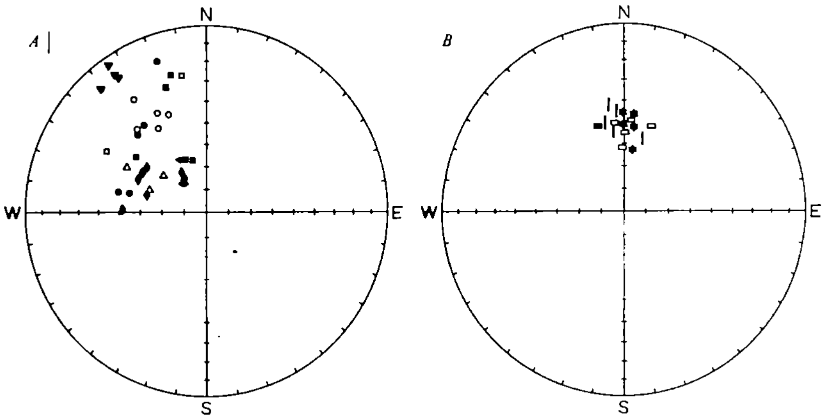

Fig. 4 Equal area projections of the site-mean directions of magnetization of the Tertiary rocks from Sardinia (see also Table 1). *a*, The Oligocene/Lower Miocene rocks; *b*, the Middle Miocene and younger rocks. All symbols are projections of the impingement points on the lower hemisphere; open symbols indicate palaeomagnetic directions which had to be inverted to impinge the lower hemisphere (reversed polarity), full symbols indicate directions with normal polarity. □, ■, Ref. 7; ○, ●, ref. 5; △, α_c andesite; ▼, α_n andesite; ◆, M1t sediments and tuffs; ◆, M1T flows; ★, T_2 flow; ▭, ▬, ref. 13; ▮, ref. 7.

counterclockwise rotation of Sardinia over an angle of approximately 50°. Because the declinations of the tuffites of M1t are intermediate, it may be argued that the rotation of Sardinia actually occurred during the sedimentation of the Aquitanian/Langhian (Early Miocene) rock sequence of Castelsardo.

In summary, the lower time limit of the rotation, presumed on palaeomagnetic grounds, seems now rather well established as Late Oligocene/Early Miocene. The Castelsardo research suggests that the minimum age of the upper limit is indicated by the palaeomagnetic direction of unit T_2. The pre-Sarravallian age of these rocks could imply that the rotation was completed before the Middle Miocene, although this is at variance with the hypothesis that the crustal block of Sardinia had not completed its rotation until the Pliocene[13].

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Climatic Implications of Total Gas Content in Ice at Camp Century

WHEN firn turns into ice through densification and recrystallization, the volume of atmospheric gas trapped as the pores close off depends in particular on the atmospheric pressure. The idea of a correlation between the total gas content and the site formation altitude of high polar ice has thus been suggested[1]. This concept received new consideration when observations of climatic changes based on the isotopic composition of ice sampled along high polar ice cores were carried out[2–4], because the variations in measured isotopic content depend not only on general climatic changes (especially temperature changes) but also on different parameters among which the site formation altitude of the ice could play an important part.

Here we present the results of twenty-five total gas content determinations (Fig. 1) on apparently unfractured samples taken from the 1,400 m deep core drilling carried out at Camp Century on the North Greenland ice sheet[5]. A careful study of the isotopic composition of this ice enabled the observation of climatic variations over more than 100,000 yr (ref. 6). The total gas content of the ice was measured volumetrically in a MacLeod gauge once the sample has been melted in vacuum and the gas phase completely extracted with water vapour trapping.

The total gas content V reduced to STP and 1 g of ice can be theoretically defined by

$$V=(1/\gamma_c-1/\gamma_i)T_0P_c/T_cP_0$$

γ_c and γ_i being respectively the pore close-off density and the pure ice density at temperature T_c, T_0 the standard temperature (273.16 K), T_c and P_c the *in situ* temperature (K) and the average pressure of the gas occlusions at pore close-off, and P_0 the standard pressure.

If the climatic conditions of the formation site (temperature and pressure) are known, the measurement of V permits a calculation of γ_c. Determinations for Byrd Station (Antarctica) and Camp Century show that the total gas content within the ice remains unchanged and that the close-off densities under present climatic conditions are practically identical (0.822 ± 0.003 and 0.823 ± 0.004) and in excellent agreement with the only other precise value that has been published[7,8]: 0.824 ± 0.004 for site 2, Greenland.

Nevertheless, in the case of Camp Century, a sudden and significant decrease in the total gas content is observed below a depth of approximately 1,100 m. This discontinuity corre-

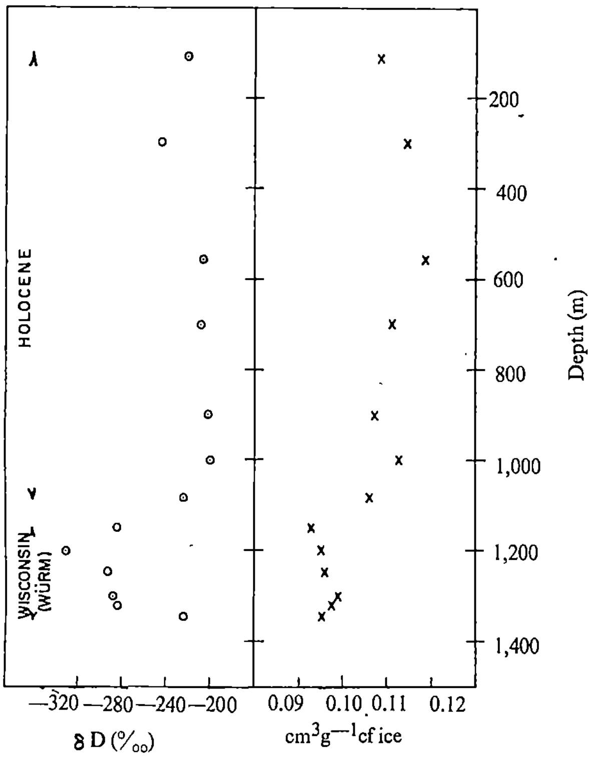

Fig. 1 Deuterium and total gas content against depth at Camp Century. Determinations of total gas content are averaged for close proximity samples.

sponds to the end of the last glaciation as is shown by the isotopic composition profile (Fig. 1) which agrees with the detailed study published by Dansgaard and others[6]. The total gas content of Wisconsin (Würm) ice is on average 12.5% lower than that of the Holocene; a decrease in temperature should have led to higher values. This decrease in the total gas content could be due to a higher close-off density during the last glaciation; alternatively, the site formation altitude of Wisconsin ice was higher than that of the present Camp Century.

Assuming γ_c has remained constant, this altitude difference would have to be about 1,300 m to account for the observed results if we use a temperature-elevation gradient of 0.9° C/ 100 m (ref. 9) and a pressure-elevation gradient of 7 mm Hg/100 m. Any general decrease in temperature linked to the last glaciation would make this difference even more pronounced. According to present ice surface elevation contours in Greenland, ice satisfying this altitude condition would have been formed at a distance from Camp Century (of the order of 800 km) which is not compatible with the present surface ice velocity (a few m per year at Camp Century). Consequently, this suggests a significant increase in the thickness of the ice sheet in this part of Greenland during the last glaciation.

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Shear Elastic Constant C_{44} and Phase Transformation of KCl

The shear elastic constant C_{44} for KCl, KF and the rubidium halides is of special interest because it decreases with pressure. Born[1] and Misra[2] pointed out that a vanishing shear elastic constant is a sufficient condition for a phase transformation. This suggestion has been elaborated by Anderson and Demarest[3] and by Thomsen[4] to postulate that the phase transformation from the NaCl to CsCl structure at high pressure may be associated with the vanishing of C_{44}. If borne out by experiment, this suggestion would be of considerable physical interest. Based on values of the first pressure derivative of C_{44} for the halides, measured at relatively low pressure, Demarest[5] extrapolated C_{44} to high pressure and showed that the phase transitions actually take place at much lower pressures than those predicted by the vanishing of C_{44}. Such extrapolation, however, does not rule out the possibility of a large downward curvature in the C_{44}/P relation at higher pressures, especially at pressures near the phase transformation leading to a vanishing C_{44}. Thus direct measurement of C_{44} up to the pressure of phase transformation appears necessary for a test of the hypothesis. I present here experimental results for ultrasonic determination of C_{44} for a KCl single crystal up to 20 kbar at which pressure it transforms from its NaCl structure to the CsCl structure.

The high pressure apparatus is of the Bridgman type[6,7], capable of generating hydrostatic pressure up to 30 kbar. The pressure-transmitting medium was 2-methylbutane. Pressures up to 4 kbar were measured by a Heise gauge calibrated against a free piston gauge. Above 4 kbar, a manganin resistance coil calibrated against the Heise gauge up to 4 kbar was used to monitor pressure. The accuracy in pressure determination is 0.1% up to 4 kbar and 0.5% at higher pressures.

The single crystal was of optical quality; the two surfaces parallel to (110) were polished flat. Two a.c.-cut quartz

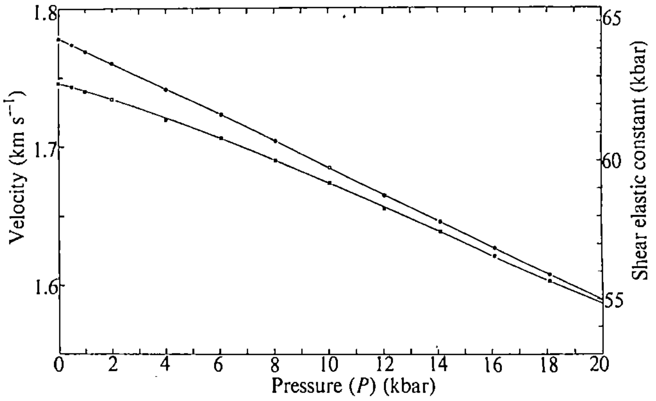

Fig. 1 Velocity (○) and shear elastic constant (□) of KCl against pressure.

transducers of 20 MHz were mounted on these surfaces to generate shear vibrations in the (100) direction. The conventional "pulse transit" technique[8] was used to determine the transit time of ultrasonic pulses through the specimen. The procedure for calculating velocity and C_{44} from the measured transit time is elementary; Murnaghan's equation of state, together with the elastic constants determined by Bartels and Schuele[9], were used to estimate sample density and the change of its dimension under pressure. Accuracy in the velocities is estimated to be 1%; relative precision among the measurements is better than 0.1%.

Both the velocities and the corresponding values of C_{44} are plotted against pressure in Fig. 1. The pressure derivative of C_{44} at 1 atm is found to be -0.31, in good agreement with the determination of Bartels and Schuele[9]. A slight downward curvature appears in the C_{44}/P relation, but this curvature does not become great enough to bring C_{44} near to zero. The relatively large uncertainty in pressure determinations above 4 kbar does not allow an accurate determination of the magnitude of this curvature. I conclude that the phase transformation of KCl from its NaCl to the CsCl structure is not associated with the vanishing of C_{44}.

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BIOLOGICAL SCIENCES

Enhanced Utilization of Brain Acetylcholine during Morphine Withdrawal in the Rat

MORPHINE prevents the release of acetylcholine (ACh) from cholinergic neurones[1–9] when the neurones are stimulated at a low but not at a high frequency. Thus a special mechanism for neurotransmitter release at low frequencies seems to be involved[10]. Although the relationship of this effect of morphine to analgesia is unclear, it may be important to the sedative actions of morphine as well as to the phenomenon of physical dependence. Some aspects of the morphine withdrawal syndrome have a cholinergic component[11–13]. Morphine reduces the release of brain ACh[1–9,14,15] so it is to be expected that its turnover is reduced in acutely narcotized animals, and enhanced during withdrawal of chronic morphine dependent animals.

Direct measurements of ACh turnover have not been made in morphine dependent animals because of methodological complexities. Large and Milton[16,17], however, using the cholinesterase inhibitor, physostigmine, provided indirect evidence for enhanced ACh turnover during morphine withdrawal in the rat. This report describes data which are compatible with their conclusion, but which were obtained using two different inhibitors of ACh synthesis. One of these, hemicholinium-3 bromide (HC-3), presumably acts by preventing choline transport across various cellular membranes[18–21]. The other, acetylseco hemicholinium-3 bromide (acetylseco HC-3), is an inhibitor of choline acetyltransferase[22]. Intraventricular (i.v.t.) injection of these compounds reduces total brain ACh. This effect is reversed by morphine and related narcotic agonists and narcotic antagonists[23–25]. The technique is not a direct measure of brain ACh turnover. It is an acceptable, indirect approach to the study of ACh utilization because (a) the expected decrease in brain ACh was observed, (b) the incorporation of ^{14}C-choline into ^{14}C-ACh was clearly reduced (Domino *et al.*, unpublished results), and (c) data obtained with pentobarbital using this method are in complete agreement with those reported for pentobarbital using incorporation of ^{14}C-choline into ACh as a measure of turnover[26,27].

Male Holtzman rats (20–30 days old) were housed in group cages in an artificially illuminated room maintained at 24±2° C. All animals were kept on an automatic 7.30 a.m. to 12.00 p.m. light and 12.00 p.m. to 7.30 a.m. dark cycle. Food and water were freely available. Morphine was administered twice daily at approximately 8.00 a.m. and 8.00 p.m. on the following schedule for the 2 week series: day 1, 10; day 2, 20; day 3, 30; day 4, 50; day 5, 60; day 6, 80; day 7, 100; day 8, 110; day 9, 120; day 10, 130; day 11, 150; day 12, 160; day 13, 180 and day 14, 200 mg kg^{-1}.

Animals given morphine for 8 weeks were on the following twice daily schedule: day 1, 20; day 2, 25; day 3, 30; day 4, 35; day 5, 45; day 6, 50; day 7, 55; day 8, 60; day 9, 65; day 10, 70; day 11, 75; day 12, 80; day 13, 85; day 14, 90; day 15, 95; day 16, 100, and 100 mg kg^{-1} for the remaining 6 weeks. Groups of at least twelve animals were run initially. There were six or more surviving animals per group. All injections were given intraperitoneally (i.p.) except in the animals receiving morphine for 8 weeks, where occasional subcutaneous (s.c.) injections were given to reduce mortality. Control animals were given 0.9% NaCl. After chronic administration of morphine for 15 or 57 days, animals were given, in the morning at the regularly scheduled time, a narcotic or saline, i.p., and HC-3 and/or acetylseco HC-3, i.v.t., and 30 min later killed by decapitation. All animals were given diethyl ether–air anaesthesia for the i.v.t. injections and allowed to recover. Animals were killed after 30 min because the rate of depletion of ACh was relatively linear at this time. ACh depletion was almost maximal with a survival rate of approximately 80%[23–25]. Total ACh was extracted from brain tissue (minus cerebellum) with the acid alcohol method of Stone[28] and bioassayed using the frog rectus abdominis method as described by Feldberg[29]. Hydrolysed tissue extracts to which ACh standard was added were used to account for the presence of sensitizing factors. All drugs were given in conveniently available salts and dosage calculated as base content. Because HC-3 and acetylseco HC-3

Table 1 Effects of Various Control Procedures, Morphine and Nalorphine on Rat Brain Acetylcholine after Acute Drug Administration

Treatment	Dose of narcotic (mg/kg)	No.	Brain ACh mean ±s.e. nmol g^{-1}	*P* value*
NaCl, 0.9%	—	8	18.7±0.4	—
Nothing	—	8	18.1±1.3	NS
NaBr i.v.t.	—	8	17.4±0.9	NS
HC-3, 20 μg	—	11	9.7±0.4	<0.001
Acetylseco HC-3, 5 μg	—	8	10.6±0.4	<0.001
Morphine	10	8	19.1±0.9	NS
Morphine	200	8	22.1±0.5	<0.01
Morphine+20 μg HC-3	10	12	17.2±1.0	NS
Nalorphine	20	8	18.8±0.7	NS
Nalorphine+20 μg HC-3	10	8	8.2±0.1	<0.001

* Group comparison Student "*t*" test to post-ether 0.9% saline treated rats.

Table 2 Effects of Morphine Withdrawal on Brain Acetylcholine after Chronic Administration of Morphine for 2 and 8 Weeks

Drug	Dose of drug (mg kg^{-1})	No.	Brain ACh mean ±s.e. (nmol g^{-1})	*P* value* A	*P* value* B
2 week tolerant					
Morphine	200	9	16.6±0.4	NS	
Nalorphine	10	10	14.6±0.5	<0.01	
48 h withdrawn	0	9	14.4±0.4	<0.01	
Morphine+20 μg HC-3	200	6	11.4±0.3	<0.001	NS
Nalorphine+20 μg HC-3	10	8	7.9±0.2	<0.001	NS
48 h withdrawn+20 μg HC-3	0	7	8.0±0.3	<0.001	NS
Morphine+5 μg acetylseco HC-3	200	8	14.7±0.3	<0.01	<0.001
Nalorphine+5 μg acetylseco HC-3	10	10	7.8±0.1	<0.001	<0.001
48 h withdrawn+5 μg acetylseco HC-3	0	10	8.0±0.2	<0.001	<0.001
8 week tolerant					
NaCl, 0.9%	0	7	16.8±0.3	NS	
Nalorphine	50	8	15.8±0.5	NS	
48 h withdrawn	0	8	11.8±0.5	<0.001	
Nalorphine—10 min until death	50	7	14.4±0.4	<0.01	

* Group comparison Student "*t*" test. In column A treated rats are compared with acute 0.9% NaCl controls (data in Table 1) except for 8 week morphine tolerant animals which are compared with 8 weeks of 0.9% NaCl. In column B the values of brain ACh after narcotic plus ACh depletor are compared with the values after ACh depletor alone taken from Table 1.

were available as the Br salt, NaBr was used as a further control.

The mean brain ACh±s.e. in nmol g^{-1} wet weight for various control procedures and treatments are given in Table 1. Control steady state brain ACh values for naive animals and animals treated with 0.9% NaCl were not significantly different. Both HC-3 and acetylseco HC-3 given i.v.t. reduced brain ACh. Morphine but not nalorphine prevented the reduction of brain ACh by HC-3. In doses of 10 mg kg^{-1}, i.p., neither affected steady state brain ACh.

The effects of giving morphine for either 2 or 8 weeks twice daily differed in several important ways from those obtained with single, acute injections. As shown in Tables 1 and 2, a single large dose of morphine caused a significant increase ($P<0.01$) in steady state brain ACh of normal animals, but not in 2 week tolerant rats. Nalorphine and 48 h abrupt withdrawal both significantly ($P<0.01$) reduced steady state brain ACh. In 2 week tolerant rats, 200 mg kg^{-1} of morphine did not prevent depletion of brain ACh by HC-3 or acetylseco HC-3 as much as 10 mg kg^{-1} in non-tolerant rats did. Brain ACh was depleted even more during nalorphine and 48 h morphine withdrawal by 5 μg i.v.t. acetylseco HC-3 than in non-tolerant rats. In 8 week tolerant rats 48 h withdrawal and 10 min after nalorphine, there was a significant ($P<0.001$) decrease in steady state brain ACh. Although these data on steady state brain ACh disagree with those of Large and Milton[16,17], the findings of decreased utilization during acute morphine and enhanced utilization during withdrawal are in complete agreement. Different strains or the sex of the Wistar rats (female) may respond differently to enhanced brain ACh turnover, some showing an increase in steady state ACh and others a decrease. The interpretation of enhanced ACh turnover during morphine withdrawal using 5 μg i.v.t. acetylseco HC-3 depends on what comparison is made. In Table 1, acetylseco HC-3 reduced the ACh level from 18.7 (or 17.4) to 10.6, a mean reduction of 8.1 (or 6.8) nmol g^{-1}. In Table 2, this inhibitor reduced the ACh level from 14.6 (in the nalorphine group) to 7.8, a reduction of 6.8; and from 14.4 (48 h withdrawal) to 8.0, a reduction of 6.4 nmol/g.

The conclusion that ACh turnover is enhanced during morphine withdrawal is valid only if it is assumed that a comparison can be made between all groups and naive controls. In any event, it is quite clear that cholinergic brain function is altered by morphine in normal, tolerant and withdrawing animals. Any theory of morphine action must take into account these dramatic changes in brain ACh and hopefully point the way to more effective treatments of narcotic dependency.

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Failure of "Dystrophic" Neurones to Support Functional Regeneration of Normal or Dystrophic Muscle in Culture

THE traditional concept that muscular dystrophy is a "primary degenerative myopathy"[1] has recently been challenged, and the suggestion put forward that the disease may in fact have a neural basis. This has been based on several independent investigations, along different lines. Dubowitz[2] observed "dystrophy-like" changes in the histochemical preparations of experimentally reinnervated muscle, and postulated that muscular dystrophy might be caused by an aberration of the normal controlling influence of the nervous system on muscle. In a series of electrophysiological studies in Duchenne and other forms of muscular dystrophy, McComas *et al.*[3-5] demonstrated a marked reduction in the number of motor units. Similarly, in the dystrophic mouse it was found that many muscle fibres were functionally denervated[6] and that there were fewer motor units, which were still of normal size[7].

In transplantation studies on dystrophic mice[8] and hamsters[9], it was observed that either normal or dystrophic muscle transplanted into a dystrophic host failed to regenerate, whereas normal and dystrophic muscle transplanted into a normal host did regenerate, the normal muscle being morphologically normal and the dystrophic regenerate dystrophic in appearance. Salafsky[10] also obtained no regeneration in the dystrophic mouse, but found that dystrophic or normal muscle transplanted into a normal host developed normal electrophysiological twitch characteristics after regeneration.

When grown in isolation in tissue culture, dystrophic human muscle had the same regenerative capacity and morphological and histochemical characteristics as normal muscle[11,12], suggesting that some additional influence *in vivo* caused the dystrophic change.

The evidence is still essentially circumstantial, and direct proof of an abnormal neural influence is still lacking. Thus, the possibility of humoral rather than neural influences affecting the regeneration of transplanted muscle in the intact animal has not been ruled out, and the electrophysiological studies cannot prove that the decrease in motor units is actually the primary lesion rather than an associated or even sequential pathological process.

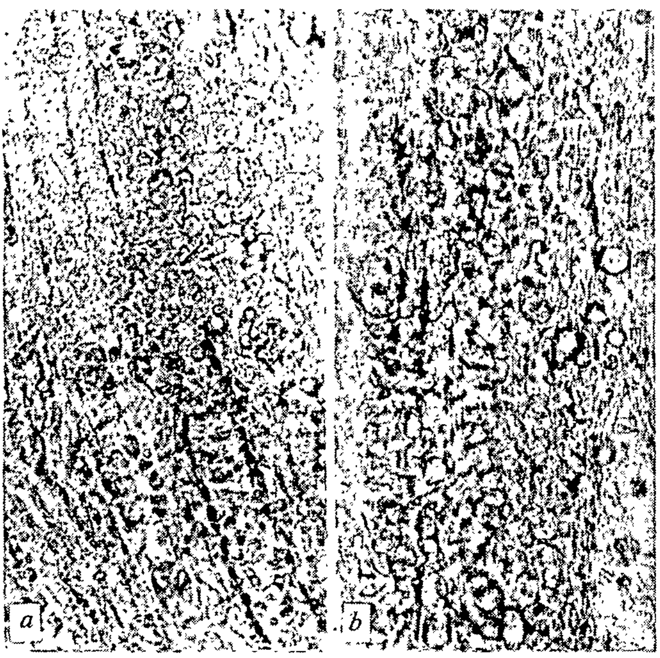

Fig. 1 Cellular regenerative phase of muscle after contact with cells from the spinal cord (to the right of the muscle). *a*, Dystrophic muscle coupled with normal cord; muscle 8 d *in vitro*; *b*, dystrophic muscle coupled with dystrophic cord; muscle 7 d *in vitro*. Unstained living cultures, ×160.

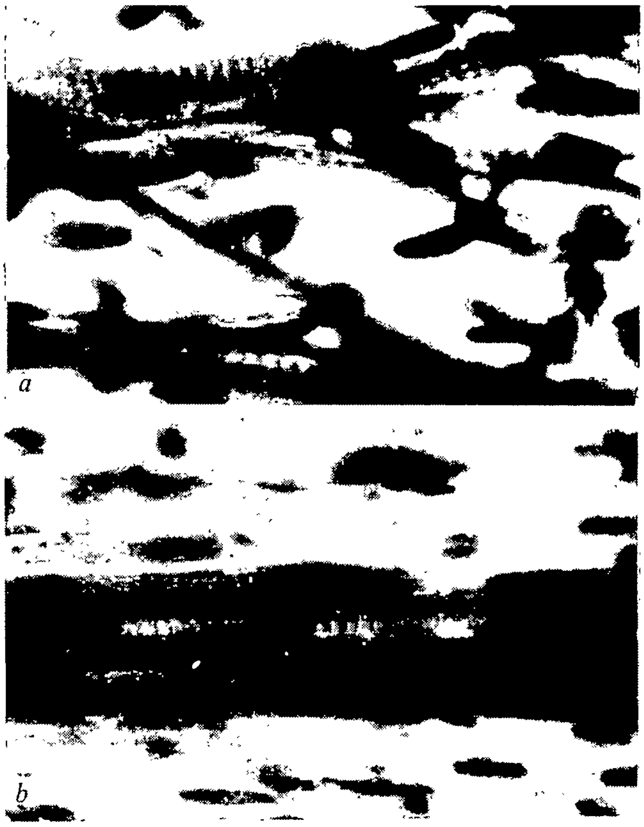

Fig. 2 Regenerated muscle fibres with well developed cross-striations, peripherally located nuclei, and spontaneous synchronized contractions. *a*, Normal muscle coupled with normal cord; 44 d *in vitro*; *b*, dystrophic muscle coupled with normal spinal cord; 25 d *in vitro*. Holmes's silver impregnation, ×800.

The tissue culture of nerve and muscle in combination has advantages over other experimental models in the study of the pathogenesis of muscular dystrophy. The system is versatile, as it is possible to grow nerve and muscle tissues of different species together and obtain functional innervation without tissue rejection[13]. The selective effects of the nerve and/or muscle can be clearly defined, as the cultures are grown away from humoral influences.

In our experiments, four series of cultures were established: normal nerve/normal muscle (ten cultures); normal nerve/dystrophic muscle (twenty-one cultures); dystrophic nerve/normal muscle (six cultures); dystrophic nerve/dystrophic muscle (seven cultures). Cultures were grown[14] on collagen-coated coverslips in Maximow double coverslip assemblies at 34°-35° C in the lying-drop position; twice a week they were washed in balanced salt solution and fed a fresh drop of medium (33% human placental cord serum, 10% chick embryo extract, 50% Eagle's MEM with 0.01 HEPES, 600 mg% glucose, and 1.3 μg ml^{-1} achromycin).

Nerve explants consisted of cross-sections of spinal cord containing one pair of ganglia, dissected from 14 to 15-d-old embryonic mice, care being taken to keep the meninges intact. Five days after explantation of the spinal cord, bundles of twelve to twenty-five muscle fibres were teased from the tibialis anterior muscle of adult mice, and positioned around the ventral side of the cord. Muscle was taken from normal mice (Swiss white) and from homozygous dystrophic mice of both the Bar Harbour strain (129/reJ-dy) and the allelic form (C57Bl/6J-dy^{2J})[15]. The clinical symptoms and histological changes are similar in both forms of dystrophy, but are less severe and of slower progression in the C57 mice[15], and homozygotes for this strain are able to mate. Normal and

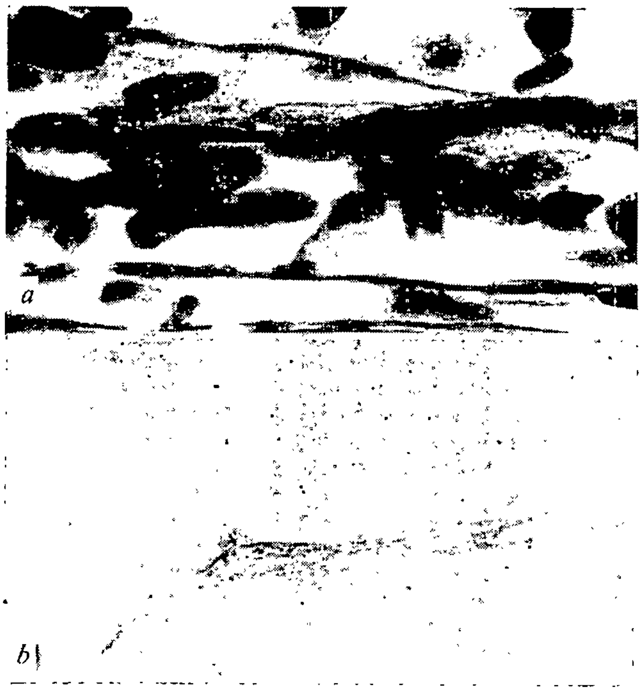

Fig. 3 *a*, Nerve ending on regenerated muscle fibre. Dystrophic muscle coupled with normal spinal cord; 28 d *in vitro*. Holmes's silver impregnation, ×800. *b*, Localization of acetylcholinesterase activity at endplate on regenerated muscle fibre. Normal muscle coupled with normal spinal cord; 30 d *in vitro*. Acetylcholinesterase, ×800.

dystrophic (homozygous) embryos were of the Swiss white and C57Bl/6J-dy^{2J} strains respectively.

Regeneration of the muscle was stimulated by contact with cells from the spinal cord. This occurred in all four combinations of normal and dystrophic cord and muscle (Fig. 1). The subsequent course, however, showed a striking difference between normal and dystrophic cord.

If contacted by ventral root neurites from normal spinal cord, the regenerated cells of both normal and dystrophic muscle then fused to form new muscle fibres (Fig. 2), with well developed cross-striations and peripherally located nuclei. These showed spontaneous synchronized contractions of groups of fibres. Muscle remained striated with good contractile activity throughout the time in culture (7 weeks). No differences in regeneration or survival could be detected between normal and dystrophic muscle innervated by normal spinal cord, except that there appeared to be slightly greater and quicker regeneration in dystrophic muscle. The formation of neuromuscular junctions was established on morphological (silver impregnation) and histochemical (acetylcholinesterase activity) grounds (Fig. 3*a* and *b*).

The behaviour of muscle coupled with "dystrophic" spinal cord was in striking contrast. The initial cellular regenerative response (Fig. 1*b*) was followed in nearly all cases (ten out of thirteen) by degeneration and disappearance of all muscle elements, leaving only strands of connective tissue. In only three cultures were myotubes formed (Fig. 4), some of which showed fibrillatory, labile, and asynchronous contractions. No cultures showed synchronized contractions of groups of muscle fibres. The nuclei of the myotubes remained in an internal position, and in only one myotube were cross-striations faintly visible (Fig. 4*b*). These myotubes bore a striking resemblance to those of uninnervated muscle in culture[11,14]. One nerve ending was observed morphologically, but did not appear to terminate on a myotube, and there was no localization of acetylcholinesterase activity.

Quantitative assessment of the regeneration of the cultures after 3 weeks *in vitro* showed that there was a significant difference in the formation of myotubes and muscle fibres between the muscle coupled with normal and "dystrophic" cord (Table 1). Qualitative differences were also observed between these myotubes of the two groups; those from muscle coupled with "dystrophic" cord did not appear to be functionally innervated, whereas those from muscle innervated by normal cord showed many of the characteristics of mature muscle fibres. Contractile activity was also qualitatively different in the two groups.

Table 1 Percentage of Cultures with Myotubes during First 3 Weeks *in vitro*

Spinal cord	Muscle	Total No. of cultures	% (No.) of cultures with myotubes	Mean % ±s.d. for group	Significance
Normal	Normal	10	90 (9)	93.5±5.8	
Normal	Dystrophic A*	11	100 (11)		
Normal	Dystrophic B†	10	90 (9)		
					$P<0.001$
Dystrophic B	Normal	6	17 (1)	23.0±8.0	
Dystrophic B	Dystrophic A	4	25 (1)		
Dystrophic B	Dystrophic B	3	33 (1)		

* 129/reJ-day; † $\text{C57 Bl/6J-day}^{2J}$

These results show two things. First, that murine dystrophic muscle behaves morphologically quite normally when regenerating in the presence of normal spinal cord; and second, that regeneration of both normal and dystrophic muscle is severely affected when coupled with spinal cord from dystrophic mice. The state of the muscle is therefore determined by that of the spinal cord. The absence of any humoral, vascular, or higher central nervous influences in this experimental model would suggest that neurones in the spinal cord are by themselves capable of producing the myopathic state of the muscle. The neurones and neuritic growth of the "dystrophic" spinal cord appeared to be morphologically normal; yet functional neuromuscular junctions did not develop. A chemical or physiological fault may thus be responsible.

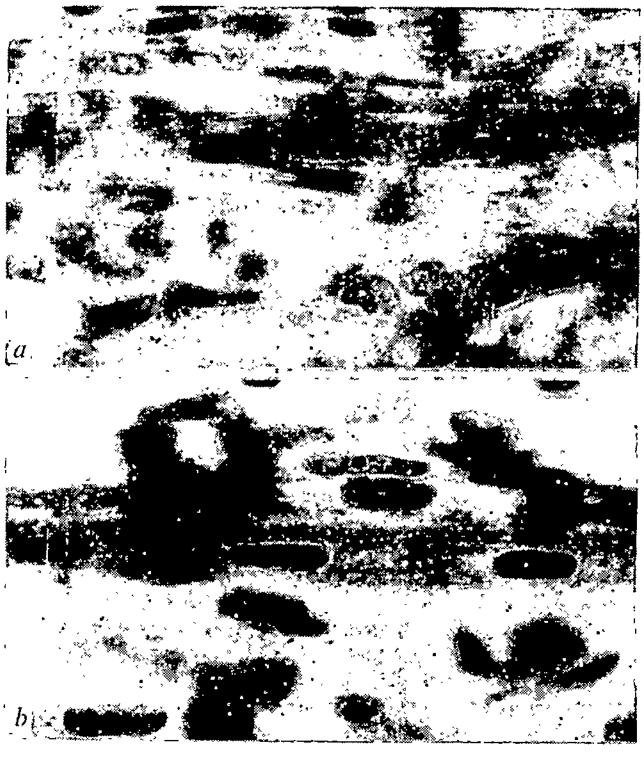

Fig. 4 Regenerated myotubes of muscle coupled with "dystrophic" spinal cord; 21 d *in vitro*. *a*, Normal muscle. Verhoeff-Van Gieson stain, ×800. *b*, Dystrophic muscle. Holmes's silver impregnation, ×800.

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Collagen Synthesis in Long-term Amniotic Fluid Cell Cultures

CYTOLOGICAL investigations indicate different possible origins of amniotic fluid cells (AFCs). The upper respiratory, gastrointestinal and genitourinary tracts, the skin, amnion and umbilical cord[1,2] are all considered to be sources of AFC. The growth of epithelioid and fibroblastoid cells is common[3] in the early stages of culture, and during further cultivation the cells are characterized by uniform fibroblastoid morphology, but their origin is not yet clear[4,5]. Our preliminary results in two human AFC lines suggest evidence of collagen synthesis in these cultures and include the examination of ratios of soluble to insoluble collagen[6].

We have studied collagen synthesis in five AFC cultures (Table 1). The AFCs were obtained by amniocenteses in the course of sectio minor and cultivated in EPL medium[7] with 10% calf serum and Eagle's minimal essential medium (MEM) vitamins[8]. After the first passages the cells were propagated in this medium without serum; the E26 cells were grown without serum from the start of cultivation. The male sex of cultivated E14 and E15 AFCs derived from male foetuses was proved by direct cytogenetic examination, by fluorescence determination of Y chromosome[9] and the F body in 34 to 40% of cells, eliminating the possibility of contamination by maternal fibroblast in the cultures under investigation.

The cells for biochemical investigation were collected in the stationary phase, 7 to 10 d after subcultivation during 10 to 24 weeks of *in vitro* cultivation in particular lines by the methods described earlier[10] (Table 1). Total collagen corresponds to the sum of hydroxyproline in isolated collagen fractions and collagen in the supernatant after centrifugation of cell suspensions obtained from AFC cultures. Soluble and insoluble collagen fractions were isolated from the cell pellet. Cell nitrogen is the sum of nitrogen in the supernatant after centrifugation of the original cell suspension, nitrogen in isolated collagen fractions and in the residue of cells after extraction with hot trichloroacetic acid (see Table 1).

During the phase of primoculture actively growing epithelioid colonies were seen, gradually exhibiting fibroepithelioid characteristics. After the first passages, they were characterized by typical uniform fibroblast morphology in monolayer cultures.

The fibroblast differentiation was proved by collagen synthesis in all lines under investigation (Table 1). The hydroxyproline/N ratio varied between 2.6 and 5.52, with the average value 3.83 except for the E8 culture, where the hydroxyproline/N ratio reached 9.13. This deviation may reflect the individual variability depending on the donor and culture derived. This possibility is suggested by finding a similarly high value for the hydroxyproline/N ratio in parallel E8 fibroblast cultures derived from foetal peripheral blood[11]. Values higher than the average range were also found for some samples from the many investigations reported previously. The observed values of hydroxyproline/N correspond well with the variation range (3.3 ± 0.3) found in other types of long term cultivated human diploid fibroblast lines[12,13]. The collagen synthesis of fibroblast cultures derived from AFC and amnion was nearly identical. The mutual proportions of soluble to insoluble collagen exhibit variability in particular lines in the range 0.44–1.43, which are in good agreement with variation range (1.23 ± 0.67) found in other types of long term cultivated human diploid cell lines[10].

The demonstration of collagen synthesis in the AFC cultures investigated identifies the long term cultivated cells as fibroblasts. Similar characteristics of collagen synthesis and maturation as in human diploid fibroblastic cell lines are evidence for the mesenchymal origin of long term cultivated amniotic fluid cells; collagen synthesis by mammalian cell lines of non-fibroblastic origin is minimal[14]. These cultures

Table 1 Collagen Synthesis in Amniotic Fluid Cell Cultures

Foetus	Age (weeks)	Sex	Length of cultivation (weeks)	Total (μg hydroxyproline/mg N)	Collagen Soluble (μg hydroxyproline)	Collagen Insoluble (μg hydroxyproline)	NSC/ISC
E2	20	♀	10	3.76	—	—	—
E2 AMN				4.34	—	—	—
E8	16	♀	14	9.13	48.3	109.5	0.44
E14	24	♂	11	2.61	4.33	3.03	1.43
E15	16	♂	12	5.52	20.3	40.7	0.50
E26	24	♀	19	2.93	8.57	7.45	1.15

Nitrogen (N) was determined by nesslerization. Hydroxyproline was ascertained by the modified Prockop and Udenfriend's method[15] after acid hydrolysis (6 N HCl, 105° C for 16 h). Soluble collagen (NSC) refers to collagen extractable at 4° C into 0.7 M NaCl, *p*H 7.2. Insoluble collagen (ISC) was extracted by hot trichloroacetic acid[16], and corresponds to collagen remaining after the extraction with NaCl. AMN, parallel culture derived from the amnion.

could possibly be used, therefore, for the prenatal investigation of hereditary disorders of human connective tissue.

The amniocenteses were carried out in the Third Department of Gynaecology and Obstetrics, Faculty of Paediatrics, Charles University, Prague.

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A New Consistent Chromosomal Abnormality in Chronic Myelogenous Leukaemia identified by Quinacrine Fluorescence and Giemsa Staining

Cells from nine consecutive patients with chronic myelogenous leukaemia (CML) have been analysed with quinacrine fluorescence and various Giemsa staining techniques. The Philadelphia (Ph^1) chromosome in all nine patients represents a deletion of the long arm of chromosome 22 (22q −)[1,2]. An unsuspected abnormality in all cells from the nine patients has been detected with these new staining techniques. It consists of the addition of dully fluorescing material to the end of the long arm of one chromosome 9 (9q +). In Giemsa-stained preparations, this material appears as an additional faint terminal band in one chromosome 9. The amount of additional material is approximately equal to the amount missing from the Ph^1 (22q −) chromosome, suggesting that there may be a hitherto undetected translocation between the long arm of 22 and the long arm of 9, producing the 9q + chromosome.

Bone marrow specimens were prepared for chromosomal analysis as previously described[3]. We cultured peripheral blood leukocytes for 24 or 48 h without phytohaemagglutinin to obtain circulating immature leukaemic cells in division, and for 72 h with phytohaemagglutinin to obtain lymphocytes in division. The time in Velban ® was reduced to 40 min for bone marrow and 60 min for peripheral leukocytes so that extended chromosomes, which are more useful for quinacrine fluorescence and Giemsa staining, could be obtained.

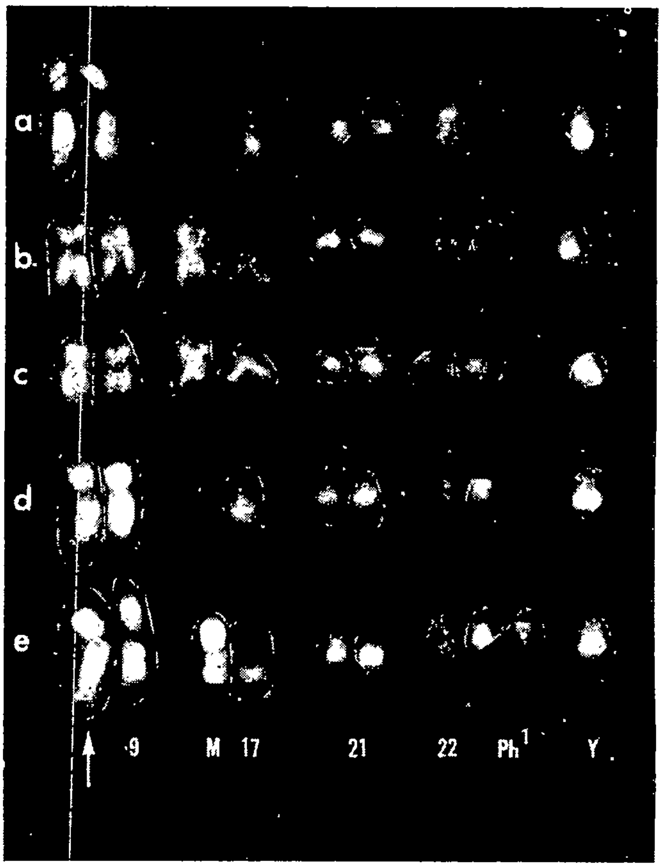

Fig. 1 Partial karyotype of five mitotic cells from cases 1–3 photographed with quinacrine fluorescence. The first pair of chromosomes in each row is the 9, with the 9q+ chromosome identified by the arrow. Note the extra band of dully fluorescing material at the end of each 9q+ chromosome. The next chromosome is the metacentric marker chromosome (M), when one is present, paired with the normal chromosome 17. The pair of chromosomes 21 is followed by the normal chromosome 22 and the Ph^1 chromosome. The Y chromosome is the last one in each row. *a*, Cell obtained from case 1. *b* and *c*, Cells obtained from case 2. The upper arm of the marker has two bands including one near the centromere, which would not be expected in an isochromosome 17. *d*, Cell obtained from case 3 before transformation. Note the relatively bright short arm region of the Ph^1. *e*, Cell obtained from case 3 after transformation. One arm of the marker appears to be homologous to the long arm of 17; the other arm shows relatively even moderate fluorescence. The two Ph^1 chromosomes are arranged with that similar to the initial Ph^1 being placed first.

Some slides were used for the initial analysis and then destained as previously reported[4], while other slides were prepared from material which had been stored for several weeks to 9 months at −20° C in fixative (45% glacial acetic acid, 55% methanol). All slides, either newly prepared or destained, were stained in a solution of quinacrine mustard (approximately 50 μg/ml.) dissolved in McIlvaine's buffer at *p*H 5.5 for 30–60 min, rinsed twice in buffer, *p*H 5.5, mounted in buffer, and examined as previously described[5]. Well-spread metaphases were photographed with a 95X fluoride objective containing an iris diaphragm on Kodak Panatomic X film using exposure times of 8–15 s. After the coverslips had been removed and the slides dehydrated through 70% and 95% alcohol, they were stained with Giemsa (Gurr's Giemsa R66) directly or after treatment either with the ASG technique[6] or with NaOH[7]. Six to fifty cells from each patient were analysed with both quinacrine fluorescence and Giemsa stain. Duplicate karyotypes were prepared according to the Chicago[1] and Paris[2] nomenclature from photographs of quinacrine fluorescence and Giemsa-stained cells in metaphase.

The patients had had CML for 2 months to 6 years, and all had responded well to busulphan. Case 2 had received prednisone and 6-mercaptopurine one year before cytogenetic analysis. Cases 2, 4, and 5 were studied only in the acute blast phase, whereas cases 1 and 3 were studied in remission as well as in the acute blast phase of CML. Case 3 showed a change in modal chromosome number from 46 in remission to 50 in the terminal phase of his disease. Cases 6 and 7 were in the early phase, and cases 8 and 9 were in remission at the time of analysis. Cultures of peripheral lymphocytes from five of the patients, three of whom were in blast crisis, have provided no mitotic cells suitable for analysis. No cultures were taken from the remaining patients. Cytogenetic findings are summarized in Table 1.

Analysis of cells from all nine cases confirmed previous reports[8,9] that the Ph^1 chromosome was a deleted 22 (Fig. 1). All cells examined with fluorescence showed that one chromosome 9 had additional dully fluorescing material at the terminal portion of the long arm (9q+) (Figs. 1, 3, and 5). Giemsa-stained cells showed an additional faint terminal band on the same chromosome 9 (Figs. 2 and 4). This change was seen in cells from case 3 obtained before blast crisis (Figs. 1*d* and 2*d*), as well as in samples obtained in the blast phase (Figs. 1*e*, 2*e*, and 3). The additional material is approximately equal to one-half the length of the moderately fluorescing region of the long arm of 9.

The occurrence of an apparently identical chromosomal abnormality in addition to the Ph^1 chromosome in nine patients with CML indicates that the chromosomal pattern in CML is not as simple as it has appeared to be. The source of the dully fluorescing and pale-staining material on the 9q+ chromosome cannot be determined at present. Its appearance is similar to that of the long arm of chromosome 22, which suggests that it may represent a translocation of the portion of chromosome 22 that is missing from the Ph^1 chromosome. In support of this suggestion, the amount of material deleted from 22 is approximately equal to the additional dully fluorescing portion observed on the 9q+ chromosome. Furthermore, other large regions of dull fluorescence, such as the end of the short arm of chromosome 1 or the end of the long arm of chromosomes 12 or 15, which could be the source of this material, appear to be intact.

The mechanism for the production of such a specific chromosomal translocation (if this is the correct explanation for these findings) is not clear. This would constitute the only specific chromosomal translocation in humans that has been

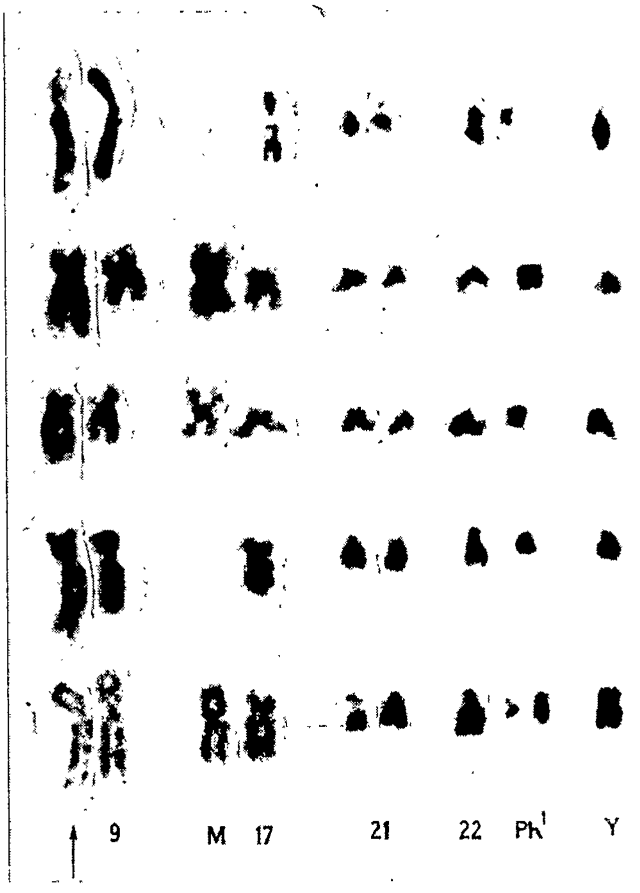

Fig. 2 Partial karyotype of the same five mitotic cells shown in Fig. 1, stained with Giemsa. The arrangement of the chromosomes is as in Fig. 1. Note the additional faint band of material at the end of each 9q+ chromosome (↑). *a*, Slide destained after quinacrine and restained with Giemsa (Gurr's R66) 1 : 10 for 10 min. There is a suggestion of banding although the preparation was not specially treated to produce bands. *b*, Slide destained after quinacrine, incubated in 2×SSC for 2.5 h, and stained with Giemsa. Note the fine band near the centromere region in the upper arm of the marker chromosome, which is not present in the lower arm. *c*, Slide destained after initial Giemsa stain and again after fluorescence; treated with 0.07 N NaOH for 1 min and incubated in 2×SSC for 2.5 h. Note the relatively large amount of centromeric heterochromatin in the marker, extending into the upper arm which may not be homologous to 17q. *d*, Slide destained after quinacrine and incubated in 2×SSC for 2.5 h before staining with Giemsa. *e*, Slide destained and incubated in 2×SSC for 2 h and stained with Giemsa. Note the similarity of bands in the lower arm of the marker with those in chromosome 17, and the longer long arm in the second Ph^1 chromosome as compared with the original.

Table 1 Summary of Chromosomal Analysis

Case	Age (yr)	Duration of CML (yr)	Source	Number of cells analysed with quinacrine and Giemsa	Karyotype*[6,7]	Figure number
1§	72	6	Marrow	8	46,XY,9q+,22q−	1*a*, 2*a*
2§	29	3½	Marrow	25	48,XY,9q+,+C,+mar,−17,+?F,22q−	1*b*,*c*, 2*b*,*c*
3§	37	3½	Marrow†	15	46,XY,9q+,22q−	1*d*, 2*d*
			Blood	26	50,XY,9q+,+8,+C,+mar,22q−, +22q−	1*e*, 2*e*
			Marrow	10	50,XY,9q+,+8,+C,+mar,22q−, +22q−	5
4§	71	1⅓	Blood	6	46,XX,9q+,+mar,−17,22q−	3*a*, 4*a*
				3	47,XX,9q+,+C,+mar,−17,22q−	
5§‡	51	2½	Blood	50	48,XY,9q+,+mar,22q−,+22q−	3*b*, 4*b*
6	45	2 mo	Marrow	6	46,XX,9q+,22q−	3*c*, 4*c*
7	25	1	Marrow	12	46,XX,9q+,22q−	3*d*, 4*d*
8	18	3	Marrow	21	46,XX,9q+,22q−	3*e*, 4*e*
9	64	3½	Marrow	11	46,XX,9q+,22q−	3*f*

* (+) additional; (−) absent; (q) long arm; (mar) marker; (22q−) represents the Ph^1 chromosome. †Obtained before blast crisis. ‡ Slides provided by Dr Lorraine F. Meisner, State Laboratory of Hygiene, Madison, Wisconsin. § Patient died in blast crisis.

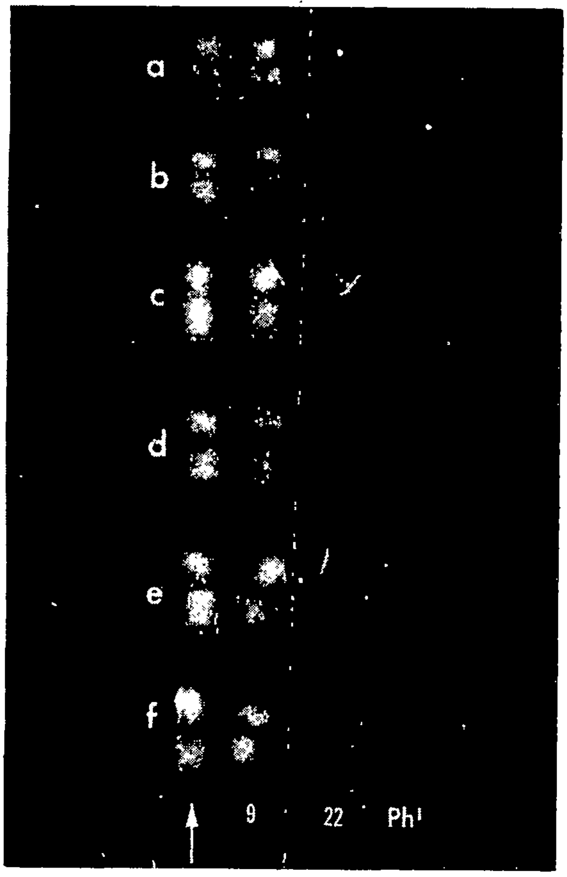

Fig. 3 Partial karyotype of six mitotic cells from cases 4–9 photographed with quinacrine fluorescence. The first pair of chromosomes in each row is the 9, with the 9q+ chromosome identified by the arrow. Note the extra band of dully fluorescing material at the end of each 9q+ chromosome. The second pair of chromosomes is the normal 22 and the Ph^1 chromosome. The cells were obtained from case 4 (*a*), case 5 with two Ph^1 chromosomes (*b*), case 6 (*c*), case 7 (*d*), case 8 (*e*), and case 9 (*f*).

identified. Previous attempts to determine whether the Ph^1 chromosome represented a loss of chromosomal material rather than a translocation floundered because it was not possible to identify precisely all the human chromosomes[10]. The 9q+ chromosome was observed in case 3 before the acute blast phase, as well as in cases 6–9 who were in remission. This suggests that the 9q+ chromosome may be present in the initial development of the somatic mutation, and that the translocation is not solely a result of progressive changes in the karyotype which frequently occur in later stages of blast crises.

Patients with CML having cells with the Ph^1 chromosome fare better clinically than patients who do not have this abnormality[11,12]. If it is assumed that the 9q+ represents a translocation from 22, then leukaemic cells with or without the Ph^1 chromosome appear to have approximately the same amount of chromosomal material. In this case, differences in the clinical course cannot be accounted for by a gross loss of chromosomal material. The assumption that the additional material on chromosome 9 comes from 22 can be tested by examination of the chromosomes from patients with CML whose cells are Ph^1 negative.

For reasons that are not understood, chromosomes from leukaemic cells are often "fuzzy" with poorly defined centromere regions. This makes accurate karyotyping of cells from leukaemic patients difficult and sometimes impossible. Nevertheless, in the present study using quinacrine fluorescence and Giemsa stains, additional findings have emerged. For example, case 3 in the terminal phase showed two Ph^1 chromosomes, but these were not identical. One consistently had a brightly fluorescing short arm region similar to that of the Ph^1 chromosome found in the analysis before blast formation. The other Ph^1 chromosome showed dull fluorescence in the short arm region and had more material in the long arm. Case 5 also had two Ph^1 chromosomes, but they were identical in size and fluorescence pattern.

Two other findings are of interest: (1) The extra C group chromosomes in two patients in blast crisis (cases 2 and 3) most closely resemble chromosome 8 in size, centromere index, and general fluorescence pattern. (2) At least one arm of the metacentric marker chromosome found in cases 2, 3, and 4 appeared to be homologous to the long arm of 17 (17q), and the marker in case 4 may be an isochromosome for the long arm of 17 (17qi). Lobb *et al.*[13] observed a metacentric marker chromosome in three patients who had CML and, with the use of the ASG technique, identified the marker as an isochromosome for the long arm of 17. The apparent frequency of metacentric marker chromosomes occurring at the time of acute blast crisis and involving the long arm of chromosome 17 remains an enigma.

As has been stated earlier[7], a combination of quinacrine fluorescence and various Giemsa staining techniques provides much more information than any one technique used separately. Application of these new techniques to the study of other types of tumours has also been fruitful. Analysis of cells from a patient with acute myelogenous leukaemia not only permitted identification of the chromosomes involved in a translocation, but also pointed to the sites of chromosomal breakage[14]. An extra terminal bright band on chromosome 14 has been observed recently by Manolov and Manolova[15] in biopsies and cell cultures from Burkitt's lymphomas in ten out of twelve

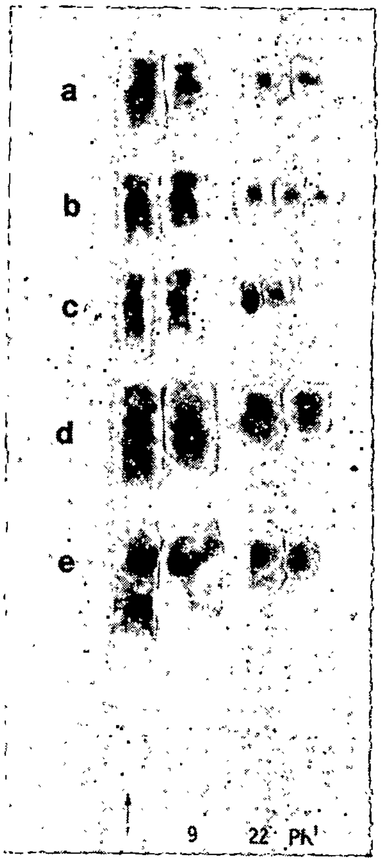

Fig. 4 Partial karyotype of five of the six mitotic cells shown in Fig. 3, stained with Giemsa. All slides were treated with 0.07 N NaOH for 2 min, incubated in 2×SSC at 60° C overnight, and stained with Gurr's Giemsa R66 1: 20 for 30 min. Note the additional faint band of material at the end of each 9q+ chromosome, and the 2 Ph^1 chromosomes in case 5 (*b*). Giemsa preparations for case 9 were unsuccessful.

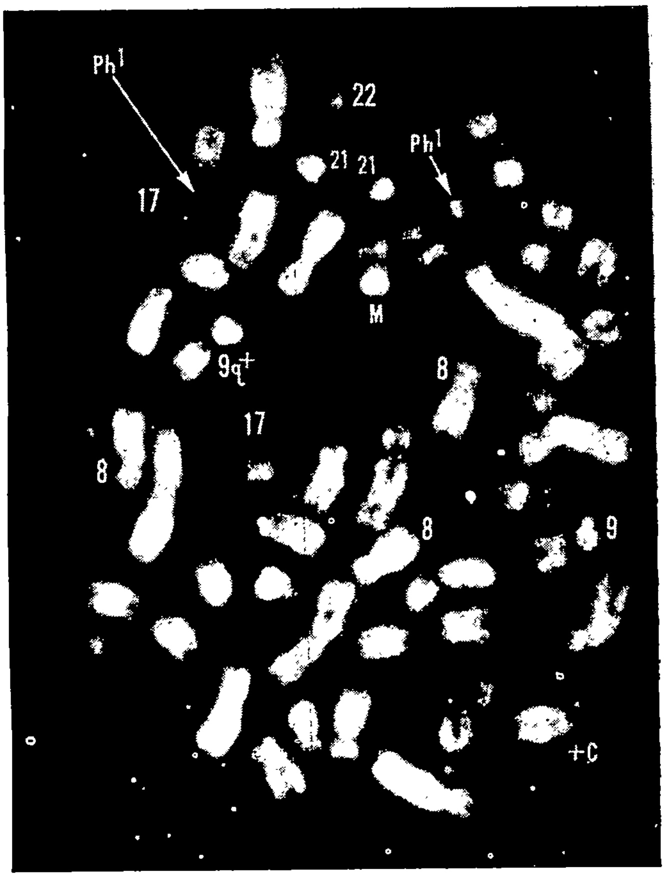

Fig. 5 Intact metaphase from case 3 stained with quinacrine mustard. This cell contains 50 chromosomes, including two extra C chromosomes, the metacentric marker, and two Ph^1 chromosomes. The 9 and 9q+ chromosomes are identified. One of the two extra C chromosomes is identical to an 8, and therefore three chromosome 8s are identified; the relative similarity in size and fluorescence pattern between the other C chromosome (labelled +C) and chromosome 8 can be observed. The arm of the metacentric marker (M) away from the label is 17q. The normal 17s are labelled, as are the normal 21s and the 22. The original Ph^1 is identified by the short arrow, and the second Ph^1 by the long arrow. In each case, the arrow points to the short arm of the chromosome. The brighter short arm and shorter long arm of the original Ph^1 chromosome as compared with the second Ph^1 are clearly demonstrated. (The difference in brightness between the two Ph^1 chromosomes seen here was noted consistently in all mitotic cells, whereas the dullness of the peripheral chromosome 17 was an artefact of this cell.)

patients. The loss of chromosome 22 in meningiomas has been confirmed with fluorescence techniques[16].

Whereas it appeared earlier from the bizarre chromosomal abnormalities identified in leukaemic cells that almost any kind of genetic imbalance might be viable, the new techniques may well demonstrate that excesses or deficiencies of only certain chromosomes can be tolerated. Careful analysis of cells from patients with leukaemia may identify certain chromosomal regions that are frequently aneuploid in leukaemic cells. This information could contribute to an understanding of the role of chromosomal changes in malignancy.

I thank Drs Stanley Yachnin, John Ultmann, and other members of the Haematology Section for assistance in obtaining samples for cytogenetic analyses, and Misses Judith Mikuta and Margaret Ikeda and Mr David Potter for technical assistance. Material from case 5 was kindly provided by Dr Lorraine F. Meisner, State Laboratory of Hygiene, Madison, Wisconsin.

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Experimental Model for von Willebrand's Disease

IT has been reported[1] that Ristocetin induces aggregation of platelets in normal platelet-rich plasma (PRP) but not of platelets from patients with von Willebrand's disease. Results presented here demonstrate that the aggregation of platelets induced by Ristocetin involves a factor present in normal and haemophilia A plasma and absent in Willebrand plasma. Lack of such a factor would then explain the inability of platelets to aggregate in response to Ristocetin in von Willebrand's disease. In eight patients fulfilling the criteria of von Willebrand's disease (Table 1), platelet aggregation induced by Ristocetin was markedly deficient when compared to healthy normal plasmas or did not occur at all. The abnormal responses of platelets from von Willebrand's patients could be corrected by the addition of small volumes of normal platelet-poor plasma (PPP) (Fig. 1); similar correction was achieved by Haemophilia A PPP but not by PPP from patients with von Willebrand's disease.

Table 1 Biological Data in Eight Patients with von Willebrand's Disease

Patients	Platelet retention %	Factor VIII activity %	Factor VIII-like antigen %	Initial velocity of aggregation to Ristocetin % 2 mg ml^{-1}	1 mg ml^{-1}
1	15	37	38	4.5	0
2	6	4	<5	0	0
3	8	10	<5	0	0
4	0	3.8	<5	0	0
5	27	10	<5	0	0
6	12.5	51.5	8	30*	3
7	0	20	<5	26.5*	0
8	0	25	<5	51*	9*
Controls	80–100	50–200	50–200	70–118	24–67

* Lag phase of 15 s.

Platelet retention to glass beads was measured by the method of Bowie *et al.*[2] and Factor VIII antigen was assayed on plasma by a modification[3] of the Laurell technique[4]. Platelet aggregation was studied in the MK III aggregometer[5] by the turbidimetric method[6] using citrated PRP. The initial velocity of aggregation was obtained by drawing a tangent to the steepest part of the aggregation curve on a potentiometric chart recorder and expressed as a percentage of the maximum transmitted light (PPP) after 30 s.

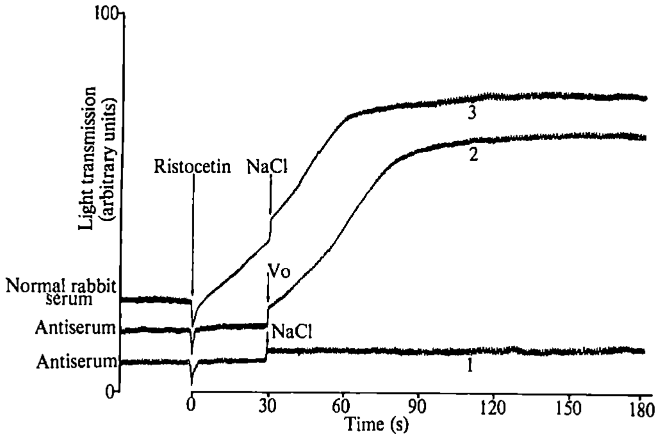

Fig. 1 Effect of normal platelet-poor plasma on Ristocetin-induced platelet aggregation in von Willebrand's disease. 0.8 ml PRP was incubated for 3 min at 37° C, with stirring, with: 1, 0.2 ml 0.154 M sodium chloride; 2, 0.05 ml normal PPP+0.15 ml 0.154 M sodium chloride; 3, 0.10 ml normal PPP+0.10 ml 0.154 M sodium chloride; or 4, 0.20 ml normal PPP; 5, 0.8 ml normal PRP+0.2 ml 0.154 M sodium chloride. 10 μl of Ristocetin (2 mg ml^{-1} final concentration) was then added.

Here we report the development of an experimental model for von Willebrand's disease, based on the inhibitory effect of a specific heterologous antiserum on aggregation induced in normal PRP by Ristocetin. The antiserum was raised in rabbits using as antigen material concentrated from the void volume (V_0) fraction of High Purity AHF concentrate[7] after gel filtration through 'Sepharose' 4B. The fraction contained both Factor VIII activity (60 U mg^{-1}) and Willebrand Factor, because it corrected abnormal platelet retention to glass beads in patients with von Willebrand's disease (ref. 8 and unpublished work by D. M.). This antiserum neutralizes Factor VIII activity of normal plasma (Titre 80 Oxford U ml^{-1}); is precipitating and specific; reveals by immunodiffusion and two-dimensional electrophoresis[9] the presence of one single component in normal and Haemophilia A plasma but not in von Willebrand plasma[3]; and decreases platelet retention of normal blood to glass beads[8].

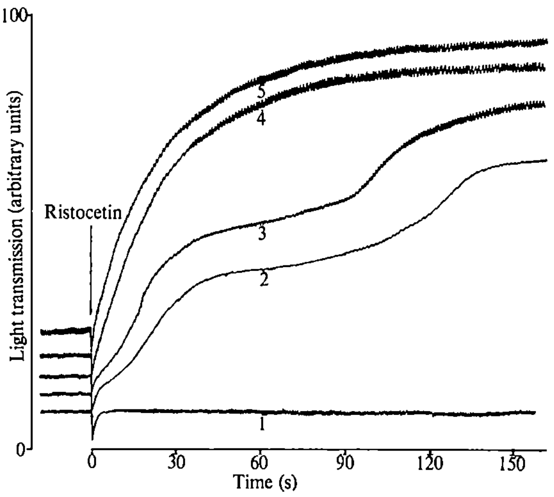

Fig. 2 Correction by the void volume (V_0) fraction of the inhibitory effect of anti-Willebrand and/or Factor VIII-like antiserum upon Ristocetin-induced platelet aggregation. Samples were incubated for 3 min at 37° C, with stirring before the addition of 10 μl of Ristocetin (1 mg ml^{-1} final concentration), followed after 30 s by 30 μl of either V_0 or 0.154 M sodium chloride. 1, 1 ml PRP+30 μl antiserum+10 μl Ristocetin+30 μl sodium chloride; 2, 1 ml PRP+30 μl antiserum+10 μl Ristocetin+30 μl V_0 fraction; 3, 1 ml PRP+30 μl normal rabbit serum+10 μl Ristocetin+30 μl sodium chloride.

The specific heterologous antiserum inhibits platelet aggregation in normal PRP caused by Ristocetin (Table 2). The degree of inhibition depends on the volumes of the antiserum added: total inhibition of platelet aggregation was achieved when 30 μl of antiserum was added to 1 ml PRP. Control experiments were performed with the use of same volumes of heterologous serum from the same non-immunized rabbit (Table 2), anti-human fibrinogen antiserum or 0.154 M sodium chloride. In no case was platelet aggregation induced by Ristocetin affected. The inhibitory effect of the antiserum was overcome by the addition of normal and Haemophilia A plasma and by the same highly purified fraction eluted in the void volume which was used to raise the antiserum (Fig. 2), but not by cryoprecipitate prepared from von Willebrand plasma or from normal or Haemophilia A plasma heated for 30 min at 56° C.

Table 2 Platelet Aggregation to Ristocetin (1 mg ml^{-1} Final Concentration) of Normal Human PRP. Effect of Anti-Willebrand Factor and/or Factor VIII-like Antiserum (Mean of Five Experiments)

Volume added (μl)	Initial velocity %	
	Control (normal rabbit serum)	Rabbit antiserum
10	41	29
20	62	32
30	56	0

Human PRP (1 ml) was incubated with normal rabbit serum or specific antiserum for 3 min at 37° C, with stirring, before addition of 10 μl of Ristocetin.

We do not know the mechanism by which Ristocetin initiates platelet aggregation. But it is different from aggregation induced by adenosine diphosphate (ADP) because aggregation by Ristocetin, unlike that by ADP, occurs in the presence of ethylenediamine tetraacetate (EDTA 0.15%)[1], is not inhibited by adenosine (5×10^{-4} M) or prostaglandin E_1 (5×10^{-4} M) but is inhibited by the specific heterologous antiserum. These observations suggest that Ristocetin-induced platelet aggregation involves a plasmatic factor known as Willebrand Factor[2,10] and/or Factor VIII-like antigen[3,8,11-13]. Thus such an experimental model can be used for further characterization of Willebrand Factor and for the study of its identity and relationship to the Factor VIII-like antigen.

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Residual Visual Function after Brain Wounds involving the Central Visual Pathways in Man

It is generally believed that total destruction of visual cortex and optic radiations in man should lead to blindness, and that regional (subtotal) destruction should correspondingly produce circumscribed areas of blindness ("scotomata") in the visual field[1]. Such areas of blindness are defined by a patient's inability to detect and report visual stimuli projected into the affected region. Standard methods of visual field testing (perimetry) suggest that such scotomata may be "absolute", that is the patient seems to be unable to distinguish between the presence and absence of visually presented targets whenever they are presented in the scotomatous area. Suspecting that the response of the visual system may depend on the task requirements, we used a technique which requires a localizing response from the patient[2], in addition to clinical perimetry. As a result, we have found evidence for the processing of information about the locus of light stimuli presented in areas of the visual field which are "blind" by the traditional definition.

Residual function in patients blinded by injury of the occipital lobes has already been established for the control of pupil size, which is known to be mediated by a reflex pathway through the pretectum[3]. Some older reports suggest that the blink reflex may still be intact in patients with complete "cortical" blindness[4,5]. Brindley *et al.*[6] reported that patients with total blindness of cortical origin can discriminate sudden changes in room illumination; ter Braak *et al.*[7] were able to elicit optokinetic nystagmus in one such patient; and Richards[8] suggested that visual information presented in a "cortically" blind area is still accessible to provide a primitive form of stereopsis. In subhuman species after lesions of the visual cortex, various residual visual capacities can be demonstrated which, however, may differ from species to species[9-14].

This report is based on experiments performed with four patients who had scotomata within their visual fields owing to traumatic (three patients) or vascular (one patient) lesions within the occipital region of their brains. Using the Tübinger perimeter[15], the visual field of each eye was carefully mapped to determine accurately the area in which the patient did not report seeing any visual target. One patient had no light perception in the right upper quadrants (A. W.), one in the left upper quadrants (L. H.), one in the right lower quadrants (W. D.), and one in the left lower quadrants with considerable involvement of the left upper quadrants (R. O.); thus, none of the patients had a complete hemianopia.

A meridian was chosen within the blind area along which visual targets were presented at various angular distances from the centre of gaze. The task of the patient was to localize by a non-verbal response the position at which the visual target had appeared; he was told to move his eyes to the position where he "guessed" the light had been presented. Patient A. W. was tested along the meridian of 30°, L. H. along 150°, R. O. along 180°, and W. D. along 330°; all patients were tested monocularly and the targets were always presented in the temporal visual fields.

In the experiment, the patient fixated in primary position a light spot subtending 10 arc min visual angle at a distance of 33 cm. This fixation point had a luminance of 100 mlambert and was projected on a homogeneous background of 1 mlambert luminance (low photopic range). The visual target whose position had to be identified was a circular white patch subtending 116 arc min visual angle and of 10 mlambert luminance; the lights were produced by tungsten bulbs. The target was flashed for 100 ms using a shutter; during this time the target moved from its initial position to a point approximately 4° further away from the fixation point. The angular separation of the target positions was either 5° (W. D., R. O.) or 10° (L. H., A. W.); no targets were presented closer than 10° from the fixation point. The sequence of target positions was random. All four patients had intact foveal vision. They had no problems in holding fixation during the experiment. Fixation could be monitored in two ways: by direct observation through a small hole in the perimeter and by the electro-oculogram which was used to record the eye movements of the patients.

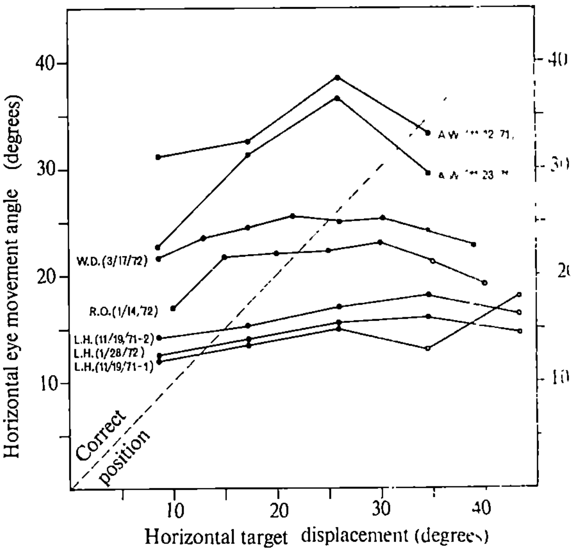

Fig. 1 Amplitude of saccadic eye movements as a function of the eccentricity of visual stimuli in patients with visual field defects owing to lesions of the occipital lobes. Targets were presented along the 30° meridian (A. W.), 150° meridian (L. H.), 180° meridian (R. O.), and 330° meridian (W. D.). R. O. and W. D. were tested once, A. W. and L. H. were tested on two different days, L. H. was tested twice on one day. The data are based only on the horizontal deviations of the eyes; a 10° eye movement along any of the oblique meridians has a horizontal component of 8.66° (10 times the cosine of 30°). Each point is the arithmetic mean of four to seven measurements. For each patient the average data points at different eccentricities are connected with each other. Note the increase of average eye movement amplitude for targets presented between 10° and approximately 30° eccentricity.

As the patients had no light perception within their scotomata, they were not able to "see" the visual stimuli to which they had to respond. Therefore, a secondary stimulus, a click from a sound source at constant position, was sounded simultaneously in order to elicit the patient's response. The click signalled to the patient that a light was being presented within his blind area and that he should move his eyes along the specified meridian to what he "guessed" was the position of the flash. He was told to move his eyes immediately, hold the deviation of his eyes for approximately 1 s, and then return his eyes to the fixation point. (For some patients this task was puzzling; one commented: "How can I look at something that I haven't seen?".) As the latency of saccadic eye movements[16] that would bring the target into the seeing field of vision is longer than the presentation time of the target (approximately 200 ms latency for visually elicited saccades compared with 100 ms for the target presentation time), the patients never reported seeing any targets during the experimental procedure.

The eye movements were recorded using standard d.c. electro-oculography. Because we were unable to obtain high quality records of the vertical components of eye movements, the data presented here are based only on the horizontal components. Calibrations were obtained at the end of an experiment by instructing the patient to look back and forth between the fixation point and continuously lit targets at different eccentricities along the horizontal meridian; these measurements were then used to determine the horizontal components of eye movements during an experiment. As all the oblique meridians used had an angle of 30° with the horizontal meridian, a target presented at 10° along any oblique meridian had a horizontal displacement of 8.66°, a target at 15° had a horizontal displacement of 12.99° and so on. The head was held on a chin rest during experimentation and calibration; a second observer verified that the patient did not move his head during either procedure.

Figure 1 summarizes the data of the experiments with the four patients. The horizontal component of the saccadic eye movements is plotted as a function of the target displacement along the horizontal meridian (abscissa). Each point corresponds to the arithmetic mean of the eye movement amplitudes for targets at any given position; the means are based on four to seven single measurements. Two patients (L. H. and A. W.) were tested on two different days, and one (L. H.) was tested twice on one day.

Clearly the patients could not identify the correct position of the targets. Each patient seemed to prefer a specific magnitude of saccadic eye movement which is similar for all target eccentricities. As is clear from the measurements in L. H. and A. W., the angle of preferred saccadic eye movements remains stable over several days or weeks.

For a given subject, if we compare the average eye position for targets closer to and farther away from the fixation point, it is evident that there is a small increase in average eye movement amplitude up to approximately 30° eccentricity along the horizontal meridian. In all seven curves in Fig. 1 the target presented at an angle of 10° from the fixation point (that is 8.66° horizontal target displacement in three patients) elicits the smallest displacement of the eye. Targets that are increasingly farther away from the fixation point lead on the average to increasingly larger eye movements; this increase is, however, limited to a central area of the visual fields beyond which eye movement magnitude (one exception) decreases again.

To evaluate the probability that the increase in average eye deviation with increasing eccentricity in the more central area of the visual field might occur by chance, some combinatorial considerations may be appropriate. For reasons of simplicity, only those eye movement amplitudes in each curve shall be considered which correspond to the three target positions closest to the fixation point. The ordering of three arithmetic means in one curve (the second point being greater than the first, the third greater than the second) has a chance probability of $1/(3!)$, that is $1/6$; as we observed the same ordering in four patients, the chance probability of our results is $1/(3!)^4$, that is $P<0.001$; and if we consider all seven curves, assuming that the curves of the same patients (L. H. and A. W.) are statistically independent, the chance probability would be $1/(3!)^7$, that is $P<10^{-5}$. The initial increase of eye movement amplitudes for targets with greater eccentricity is thus highly significant according to statistical convention.

In addition to these combinatorial considerations, a χ^2 analysis of the same data was performed. An analysis of variance could not be performed because of the inhomogeneity of the data (different meridians tested; different range of eye movement amplitudes for each patient). For each of the seven experiments the arithmetic mean of all single measurements for the three target positions closest to the fovea was determined. Then we counted the eye movements with an amplitude above or below the arithmetic mean, separated according to the three target positions. The values of the seven experiments were then added and analysed using the χ^2 technique. Under the null hypothesis (no dependence of the eye movement amplitude from the eccentricity of the targets) one would expect an equal number of eye movements above and below the arithmetic means for the three target positions. The χ^2 analysis, however, rejects the null hypotheses ($\chi^2=9.83$; $df=2$; $P<0.01$) and suggests a significant result which means that there is indeed a systematic increase of the amplitude of eye movements with an increasing eccentricity of the targets for the three target positions closest to the fovea.

In control experiments we tested whether the observed effect was due to intensity discrimination of scattered light. Two patients with retinal scotomata tested in the same experimental conditions as the patients with cortical scotomata showed no increase in eye movement amplitude for more eccentric targets. Targets with different intensities (1, 10 or 100 mlambert) presented at a fixed position (10° eccentricity) to two patients with cortical scotomata did not lead to eye movements whose magnitudes were correlated with the intensity of the flashes. Thus, stray light is probably not responsible for the observed effect. When the auditory stimuli were not accompanied by visual stimuli ("catch" trials), the magnitude of saccadic eye movements was unrelated to the sequence of target positions used in the actual experiments. It is interesting to note that the patients had no feeling about the presence or absence of light stimuli; actually they believed that their performance was always completely random.

We propose that our observation of an increase in the amplitude of saccadic eye movements for more eccentric targets presented within the "cortically" blind areas of the patients' visual fields is mediated by some still functional central visual mechanism. Perhaps this effect is due to the midbrain in these patients; the midbrain is known to receive a substantial projection from the retina. Alternatively, there may remain in the visual cortex a representation of the visual field which is not revealed by ordinary perimetry but which is still accessible to a non-verbal response, that is orienting. If so, then our evidence would indicate an unsuspected form of dissociation of function.

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Lithium Secretion by the Salt Gland of the Goose

THE mechanism by which the salt gland of marine birds secretes a hypertonic solution of sodium chloride is of considerable interest. It is generally considered that the concentration gradient for sodium is established across the luminal membrane of the secretory cell by an electrogenic sodium pump[1], but Hokin[2], on the basis of measurements of the intracellular composition of salt gland slices *in vitro*, argued that the concentration gradient is established across the basal membrane. One of us (M. P.)[3] repeated these experiments, however, and was unable to confirm Hokin's figures for the sodium and chloride content of salt gland cells. M. P. again proposed that sodium is actively transported from the intracellular fluid of low sodium concentration to the lumen of the tubules. Nevertheless, this does not preclude the possibility suggested by Hokin[2] that sodium and chloride pass into the cell across the basal membrane by an active mechanism involving exchanges for H^+ and HCO_3^-; indeed, current evidence favours this view[1].

Although Li^+ will stimulate membrane "transport" ATPase and active Na^+ sodium extrusion in some tissues[4-6], it is itself not transported by the sodium pump in the same direction as Na^+ (refs. 5, 6). In other tissues, for example, frog skin[7], toad bladder[8] and frog muscle[9], there are reports that Li^+ can be pumped, to some extent, in the same direction as active Na^+ movements. The movement of lithium in the avian salt gland, which can transport sodium at a very high rate, has therefore been investigated in order to determine (i) whether this ion is secreted and concentrated by the gland, (ii) where the electrochemical gradient is established, and (iii) if this site is similar to that for sodium. In view of the current interest in lithium as an agent of use in the treatment of psychological disorders, the results may be of wider interest.

In most studies experiments have been done *in vitro* and lithium has been added largely to replace sodium in the external medium. By infusing relatively low concentrations of lithium into the blood stream the normal external ionic environment of the cells is maintained and it seems that, at least for the periods used in the experiments described here, movements of lithium can be studied alongside those of other substances.

The methods used for studying salt gland secretion in conscious domestic geese (*Anser anser*) have been previously described[10-12]. The four birds used in our experiments had catheters inserted into a brachial artery and a brachial vein. Salt gland secretion was induced by the intravenous injection of 0.5 M NaCl (18 ml kg^{-1} body weight). When secretion was established 0.5 M LiCl was infused intravenously at a rate of 1 ml min^{-1}; blood samples were taken from the arterial catheter. The LiCl infusions were discontinued after 25 min because in earlier experiments it was found that if LiCl was infused for longer the birds became lethargic and eventually vomited. Nevertheless this period of time was sufficient for plateau concentrations of Li in arterial plasma to be reached.

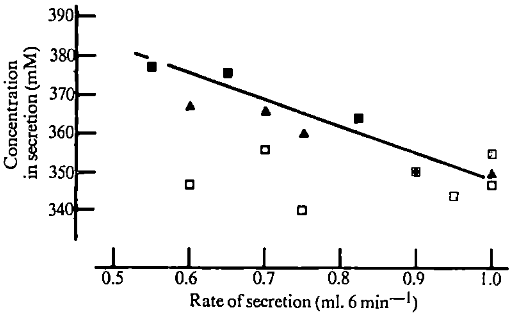

Fig. 1 Relationship between $[Na^+]$, $[Na^+ + Li^+]$ and rate of secretion in a goose before and during the infusion of 0.5 M LiCl (1 ml min^{-1}). Salt gland secretion was induced by the injection of 0.5 M NaCl (18 ml kg^{-1}) intravenously. The regression line shown was calculated from the points obtained before the infusion of LiCl. (■), $[Na^+]$ before infusion; (□), $[Na^+]$ during LiCl infusion; (△), $[Na^+ + Li^+]$ during LiCl infusion. Note that it was not possible to calculate a line for $[Na^+]$ or $[Na^+ + Li^+]$ when LiCl was being infused since plasma $[Li^+]$ was changing as a result of the infusion, and only the point at 0.6 ml per 6 min was obtained when equilibrium concentrations in plasma had been reached.

In two experiments ^{14}C-sucrose in 0.154 M NaCl was infused intravenously at 1 ml min^{-1} starting 10 min before the infusion of LiCl, in order to determine the extracellular space of the salt glands. These birds were killed by injecting 600 mg of pentobarbitone sodium intravenously during the final minute of the LiCl infusion; the salt glands were then quickly removed for analysis. The analytical methods have been described previously[3,11]. Li in plasma, nasal fluid and tissue digests was determined by atomic absorption spectrophotometry; allowance was made for interference by sodium.

In all four experiments the concentration of Li^+ in the secretion was higher than that in the plasma when equilibrium has been reached, the secretion/plasma ratio varying from 1.29 to 2.26 (Table 1). In the two experiments in which ^{14}C-sucrose was also infused in order to enable measurement of extracellular space, the concentration of Li^+ in the non-extracellular tissue space, that is, intracellular and lumen (transcellular) spaces, was calculated to be 13.3 and 6.6 mmol l^{-1} water. As the concentrations of Li^+ in the secretion were higher than these figures (15.8 and 15.3 mM), this procedure slightly overestimates the true intracellular concentration.

The concentration of Li^+ in the secretion was higher than that of the intracellular fluid, and the lumen of the ducts of the gland is electrically positive with respect both to the inside of the secretory cells and to blood[13]. So it must be concluded that Li^+, like Na^+, is actively transported against both electrical and chemical gradients across the luminal cell membrane. There is certainly good physiological evidence to suggest the presence of a Na^+ pump at this site[1] and it seems likely that Li^+ is accepted and transported by this pump. This view is strengthened by the effects on the sodium concentration of the nasal fluid when lithium is being transported. In any one bird there is an inverse relationship between the Na concentration of the secretion and the rate of secretion[11]. Fig. 1 shows that when Li^+ was being infused and transported, the figures for $[Na^+]$ fall below the line relating rate to concentration. When the values for $[Na^+]$ and $[Li^+]$ are added together, the

Table 1 Lithium Concentration in Plasma, Nasal Fluid and Intracellular Fluid (ICF) of Goose Salt Glands during the Infusion of 0.5 M LiCl Intravenously (1 ml min^{-1})

Goose	Plasma		ICF (mmol l^{-1} water)		Secretion		Secretion/plasma ratio		Secretion/ICF ratio	
	$[Na^+]$	$[Li^+]$	$[Na^+]$	$[Li^+]$	$[Na^+]$	$[Li^+]$	$[Na^+]$	$[Li^+]$	$[Na^+]$	$[Li^+]$
1	157	7.0	35	13.3	410	15.8	2.51	2.26	11.7	1.19
2	166	8.4	20	6.6	484	15.3	2.92	1.82	24.2	2.32
3	154	8.9	—	—	374	16.3	2.43	1.83	—	—
4	164	15.6	—	—	347	20.0	2.12	1.29	—	—

The figures given are those obtained for a 6 min period when a plateau concentration of Li^+ in plasma was reached. The secretion/ICF figures have been calculated on the assumption that both Na^+ and Li^+ are freely distributed in the ICF.

points fall much closer to the line obtained before the infusion of LiCl was begun. This implies that Li$^+$ and Na$^+$ are transported by the same mechanism. The affinity of the luminal pump, however, at the concentrations of lithium used in these experiments, seems to be very much greater for Na$^+$ than Li$^+$.

As far as transport into the cell across the basal cell membrane is concerned, the movements of Li$^+$ give no clue to the mechanisms involved. In the absence of active Li$^+$ transport out of the cell, [Li$^+$] would be expected to reach higher concentrations in the cell than in plasma because of the potential difference, and it is not possible to distinguish between passive Li$^+$ entry and possible Li$^+$–H$^+$ exchange[1].

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Malarial Parasites in Mammalian Platelets

It is generally accepted that mammalian plasmodia undergo their schizogonic cycle in red cells and hepatocytes. With the exception of macrophages, which often engulf parasitized red cells, and granulocytes, which rarely phagocytize parasitic debris, other cells of blood and bone marrow do not carry plasmodia.

Our previous studies in man showed a significant and consistent increase in the mean size of blood platelets during acute infection with *Plasmodium vivax* or *P. falciparum*[1]. Those studies led to a prospective, systematic analysis of the morphology and function of platelets (thrombocytes) during and after spontaneous human malarial infections. A similar study was performed experimentally using the NK65 strain of *Plasmodium berghei* inoculated intraperitoneally into young albino ICR mice.

Samples of infected blood were obtained without anticoagulant (from venous blood in man and from cardiac blood in mice) and fixed immediately in buffered, 1.5% glutaraldehyde solution prewarmed at 37° C. After fixation had occurred, platelets were separated and concentrated by differential, two stage centrifugation. A pellet of platelets was prepared using plasma poor cells as in a matrix. After postfixation in osmium tetroxide the material was embedded in epoxy and ultrasections were further stained by uranyl acetate and lead citrate.

Apart from functional abnormalities the human thrombocytes showed multiple ultrastructural changes before antimalarial therapy was instituted. In several of these thrombocytes there were large membrane-bound inclusions of granular or lamellar osmiophilic material, sometimes resembling myeloid bodies. These inclusions were suggestive of degraded parasitic material, but I was unable to identify obvious parasites in the platelets of more than eighteen patients examined by electron microscopy.

The thrombocytes of the infected mice also had inclusions of the type seen in humans. I also found several obvious parasites within mouse platelets. Some parasites could be recognized as merozoites (Fig. 1) or trophozoites; schizonts were not found. As judged by morphology alone, some of these intrathrombocytic parasites appeared to be viable. Others showed different stages of degeneration, which often resembled the inclusions described above in mouse and human platelets. The parasitized platelets were only moderately enlarged. The organelles, such as granules, mitochondria, canaliculi and microtubules, were generally well preserved and could be easily recognized, although not necessarily in all sections.

To my knowledge plasmodia, or any other protozoa for that matter, have not previously been found in mammalian platelets. The nucleated thrombocytes of some non-mammalian vertebrates may be invaded by plasmodia: Zuckerman observed by light microscopy thrombocytes, heterophils, macrophages and lymphocytes invaded by *Plasmodium gallinaceum* in one chick embryo infected in experimental conditions[2]. Intrathrombocytic parasites have also been described in saurian malaria by Thompson and Huff[3] and by Telford[4]. Scorza has observed, by light and electron microscopy, sexual and asexual stages of *Plasmodium tropiduri* in the thrombocytes of some naturally or experimentally infected iguanas (*Tropidurus torquatus*)[5]; his description suggests that a complete schizogonic cycle of *Plasmodium* may occur in those nucleated "platelets".

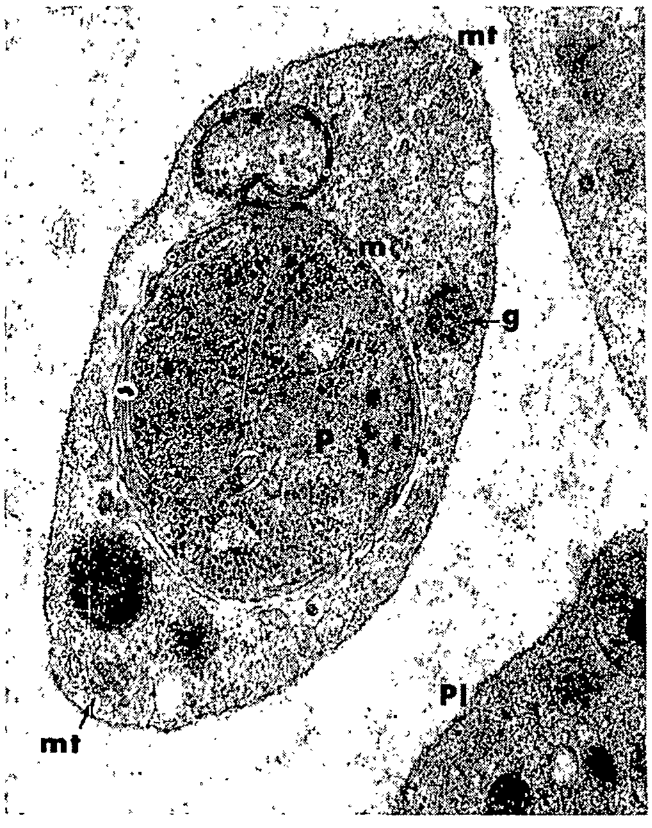

Fig. 1 Perpendicular section of a mouse platelet 2 weeks after intraperitoneal inoculation with *Plasmodium berghei*. Two granules (g) are present in the periphery of this thrombocyte. Circular profiles of the characteristic ring of microtubules (mt) are seen at the equator of the platelet. A plasmodium merozoite (P) is present in the centre, sharply separated from the platelet cytoplasm by concentrical membranes (m). The nuclear mass (left) occupies a large portion of the parasite. Dense free ribosomes are scattered in the cytoplasm. A vesicular structure, lined by a double membrane, is near the centre. The cells partially shown in the right corners (Pl) are also platelets. Uranyl acetate and lead citrate, ×40,250.

Parasites may reach the inside of the platelets either by active invasion—such as occurs when the conoid region of the merozoite penetrates the red cell—or through phagocytosis by the platelet. There is evidence that platelets are capable of engulfing such materials as thorotrast granules[6], india ink[7], starch[8], and so on; masses of the size of these parasites have not, however, been seen in mammalian thrombocytes. As the platelets and parasites are fixed immediately after the blood is obtained, this finding cannot be the result of an *in vitro* phenomenon. It is unlikely that the parasites penetrate the cytoplasm of megakaryocytes and are then released within the individual platelet segments; I have examined a large number of sections of megakaryocytes in infected mice, and a few in humans, without finding any suggestion of parasites or inclusions.

The marked platelet enlargement proved in humans[1], and often seen in mice, is probably not the direct result of platelet invasion by parasites. The larger platelets, although abnormal by electron microscopy, do not show evidence of parasitic invasion. More likely the parasitic invasion of platelets could contribute to platelet depletion, which in malaria infections seems to be caused chiefly by disseminated intravascular coagulation[9,10]. In turn this platelet depletion stimulates thrombopoiesis in the bone marrow with the release of large "young" thrombocytes[1,11].

This finding is too recent to speculate on its significance. By itself it indicates only that in this experimental mouse infection plasmodia can be found in platelets, By analogy it suggests that the same might occur in man.

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Head Movement of Flies during Visually Guided Flight

MOST visually advanced animals are able to move their eyes relative to the body. This makes vision independent of body movements; in man it enables the eyes to keep the visual world stationary on the retinae, to scan the field of view, and to maintain fixation on moving objects[1]. The extent to which animals other than vertebrates are able to perform these activities is largely unknown; many, especially arthropods, make body movements tending either to stabilize the visual surroundings[2,3] or to cause fixation of objects by the eyes[4,5]. In a very few cases, however, it has been shown that arthropod eyes may make directed movements independent of the body: examples are the fixation of prey by mantids[6] and by salticid spiders[7], and compensatory eye movements in a moving visual surround by crabs[8] and locusts[9]. Here I describe the head movements (and therefore, in insects, eye movements) made by blowflies during sustained flight. I show these to be of two kinds: rapid saccadic flicks in which the head takes up a new angle with respect to the visual surroundings, and stabilization movements which keep the angular position of the head almost constant, in spite of body movements of up to 10° in either direction.

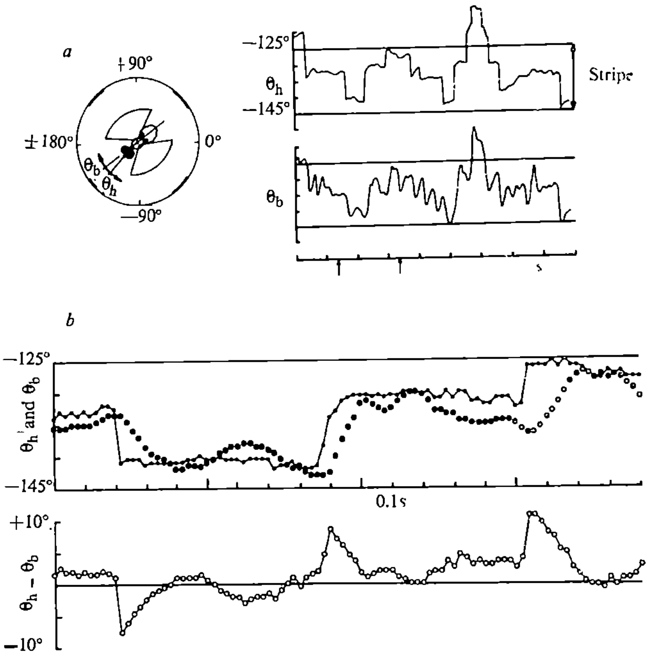

Fig. 1 *a*, Record of the movement of the head (θ_h) and body (θ_b) of a fly during a 9 s period in which it was fixating one of the four stripes of the drum, as shown on the left. The record shows 14 saccadic movements of the head. The sign convention used in all figures shows clockwise angles as negative. *b*, Part of the record from (*a*), between arrows on the time scale, at 10 times the temporal resolution. ●, Body position; ●, head position; ○, movements of the neck (θ_h–θ_b). Each point is the position on a single frame of film. It can be seen that between saccades the head position is kept stable in spite of body movements by active movements of the neck.

Blowflies (*Calliphora erythrocephala*) were suspended on a pivot which allowed them to rotate freely but prevented forward motion. The pivot consisted of a pin revolving in a narrow glass tube, sealed at the base and partially sealed at the top to prevent lateral play. A second pin was waxed to the dorsal thorax, bent around the animal and joined to the pivot. Although nearly frictionless, the pivot does add an inertial load tending to reduce the rate at which the fly can change direction. Flying flies were placed at the centre of a 12 cm diameter white drum containing four equidistant black stripes, each 20° wide by 110° high. The drum was lit from above giving an average background brightness of about 200 cd $\times\,\mathrm{m}^{-2}$. The flies were filmed from above at 50 frames s^{-1}; the films were analysed frame by frame to give the angular positions in the horizontal plane of the head (θ_h) and body (θ_b) separately. A small piece of celluloid attached to the head made it possible to measure both angles to an accuracy of about 1°.

In a stationary drum blowflies flew in the direction of one or other of the four stripes for about 75% of the time, so that θ_b was usually within 20° of the centre of a stripe; shifts of fixation from one stripe to another were frequent. Similar behaviour has been demonstrated by Reichardt in *Musca* in an artificially produced "closed loop" environment[4,10]. Although the fly's body points in the general direction of a stripe, its head movements are much more precise. Fig. 1 shows on two time scales the movements of head and body during part of a long bout of fixation of a single stripe. The following points emerge. (i) The head fixates different positions within and just outside the stripe, and maintains them accurately in spite of movements of the body. In some records the body departed by as much as ±7° from its average direction of flight, whereas the head moved through only ±2°. (ii) Changes in fixation point are accomplished by rapid saccadic movements of the neck (three are shown in Fig. 1*b*). These have amplitudes between 5 and 20°, and are complete in 20 to 40 ms. The head usually moves at at least 500° s^{-1}. (iii) After each saccade the body changes direction slowly, coming into line with the head again after 100 to 200 ms, though this is likely to be less without the inertial load of the pivot. The body then follows the same course as the head, but with less precision and some oscillation. (iv) The stability of the head between saccades is achieved by active neck movements in the opposite direction to the body movements. Thus in Fig. 1*b* the neck movements (θ_h–θ_b) are almost the mirror image of the body movements (θ_b) except during saccades.

The stabilization of the head between saccades is under direct visual control. This is demonstrated in Fig. 2 in which the drum is rotated; the head now accurately follows the drum movement between saccades as though "clamped" to the visual field.

When a fly has abandoned one stripe and is turning towards another, the behaviour of the head is similar to that during stripe fixation (Fig. 3). It makes rapid saccades with short (50 to 200 ms) intervals between them. The saccades tend to be more frequent than during stripe fixation, and are usually all in the same direction until another stripe is reached.

The combination of rapid saccadic eye movements followed by periods of relative stability of the fixation point appears to be a visual strategy common to both flies and man, and no doubt to many other visual animals. If certain kinds of perception such as pattern discrimination or the detection of external movement can only occur when the visual world is temporarily still, then it is necessary for a moving animal to have mechanisms for keeping the eyes in a constant optical relationship to the external world (stabilization), and for changing this relationship at intervals (saccades) as new information is required or locomotion shifts the scene.

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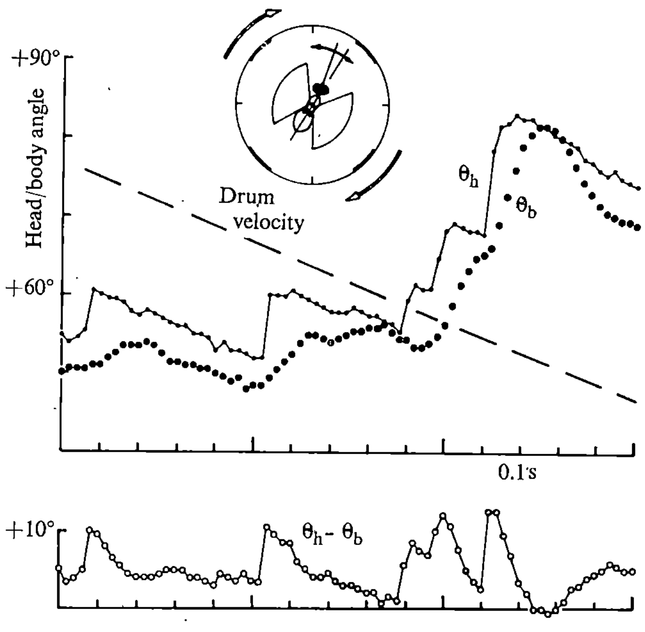

Fig. 2 Record of head and body movements, as in Fig. 1*b*, but in which the striped drum is moving clockwise at 20° s^{-1}. The dashed line indicates the drum velocity, though not the exact position of any one stripe. Between the five anti-clockwise saccades made by the fly, the head remains accurately stabilized on the moving pattern.

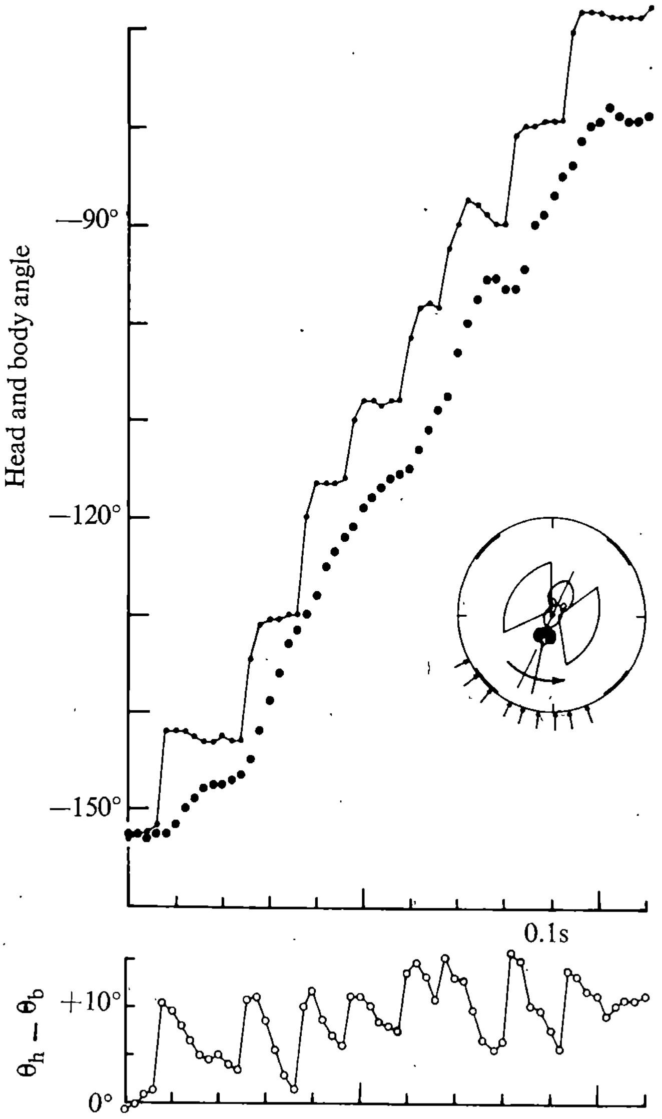

Fig. 3 Head and body movements during anti-clockwise turning when no stripe is being fixated. As in fixation, the head makes saccadic flicks with short periods of stability between them (arrows on inset). The drum is stationary.

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"Electromotor" Response in the Electric Fish *Eigenmannia* (Rhamphichthyidae, Gymnotoidei)

VARIOUS species of fish are known to emit and perceive weak electrical signals[1–4]. Whereas the physiological mechanisms involved in the production and perception of such signals have been investigated in detail (ref. 3, pages 347 and 493), less is known about their behavioural relevance. Lissman[2] proposed and gave evidence for a role in object detection, by the distortion of the fish's own electrical field due to either dielectric or conducting objects within range. This idea has been supported by later work (refs 4–6 and personal communication from A. Kalmijn and R. Adelman). The study reported here was undertaken as a first effort to devise ways of measuring the performance of the object detecting system in respect to its discrimination of particular parameters such as distance, size and motion. A primary goal was to find methods based on built-in responses, rather than time-consuming conditioning procedures, which could be used on any individual on a given day to assess its skill in relation to species norms and would thus facilitate the quantification of changes in performance after nerve transections, brain lesions and such like.

Fish of the genus *Eigenmannia* hover in plant thickets during the day and swim into open water only at night[7]. When kept in aquaria without vegetation *Eigenmannia* readily hovers in bundles of plastic strips hanging from the water surface. When a plastic arrangement of this kind is moved gently *Eigenmannia* tries to maintain its relative position inside. To determine to what extent electrical object location could account for this behaviour the following experiment was performed.

A specimen of *Eigenmannia* (resembling the species *Eigenmannia virescens*) was blinded to exclude visual cues and placed in a bare all-glass aquarium 90×30×40 cm high. Three vertical 'Plexiglas' strips, 1.5 mm thick and 35 mm wide (see cross-section in Fig. 1), were hung at the centre of the aquarium reaching down into the water a few millimetres above the glass bottom. This arrangement was chosen after six blinded specimens tested in various arrangements of 'Plexiglas' strips were found to hover preferably with the nose close to one strip and at least one side of their body near another strip. This behavioural preference was met by the symmetrical arrangement shown in Fig. 1. After 2 d of restless swimming following the operation the fish began to hover inside the 'Plexiglas' arrangement close to the bottom, usually oriented as in Fig. 1. When the fish happened to be sufficiently stationary the entire 'Plexiglas' arrangement was slowly swung horizontally in a sinusoidal manner, parallel to the plane of the strips in order to minimize water turbulence. As the 'Plexiglas' arrangement began to swing, the fish responded by translating itself right and left, still hovering, by means of its constantly undulating anal fin which tilted left and right respectively. The fish thus maintained its position relative to the 'Plexiglas' strips for several cycles (Fig. 1) until it eventually swam away. Commonly it hovered and swung back and forth over periods of 30 s to 5 min.

To determine the extent to which mechanical cues of the swinging 'Plexiglas' arrangement could have accounted for this response, an identical arrangement was built with agar strips of conductivity equal to the aquarium water (2 kΩ cm^{-1}), agar recipe developed by Kalmijn and Adelman (personal communication). Whereas the fish usually avoided contact with the 'Plexiglas' strips it now frequently bumped into the agar strips. It rarely hovered in the neighbourhood of the agar arrangement and generally had to be prodded into it before experiments could start. When the agar arrangement was swung the fish did not respond and usually escaped when eventually hit by one of the strips (Fig. 1). This suggests that *Eigenmannia* perceived the movement of the previous 'Plexiglas' strips electrically rather than mechanically. *Eigenmannia* thus follows electrically-detectable objects in a manner comparable with the way visual animals follow light patterns, for example, a beetle following the rotation of a striped cylinder. By analogy with this famous "optomotor response"[10] one can call the behaviour in *Eigenmannia* an "electromotor response".

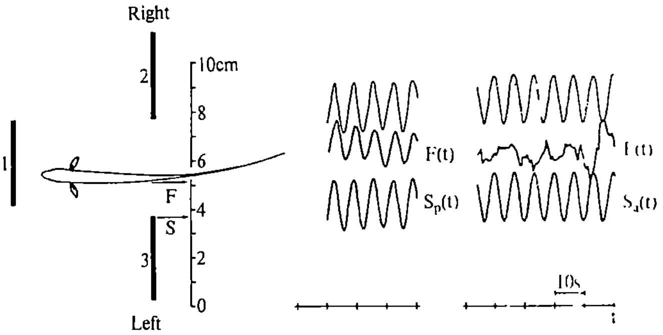

Fig. 1 Left, cross-section through experimental arrangement of three vertical strips, 1, 2 and 3, with fish hovering between strips 2 and 3. While the entire arrangement is swung right and left, its momentary position along the cm scale is recorded by the location of the inner edge of strip 3 (S). The position of the fish is recorded by the location of its left side body wall on the level of strips 2 and 3 (F). Right, two continuous records of S and F obtained by single frame film analysis. A simultaneous tracing of the inner edge of strip 2 is shown above each record. S_p, swinging 'Plexiglas' strips inducing strong response in the fish (gain=0.7, phase= $-\pi/10$ according to Fourier analysis of F and S_p at stimulus frequency). S_a, swinging agar strips of same conductivity as water fail to elicit a response (gain=0.1, phase= $-\pi$ at stimulus frequency) and cause flight as fish is hit twice at the end of the record.

The electromotor response in *Eigenmannia* shares obvious advantages with the optomotor response by being non-habituating and thus easily repeatable. Experiments can be performed almost any time with the only restriction that the animal has to be hovering rather than restless. A healthy and well fed animal, however, can be expected to hover for at least a few hours every day, whereas specimens suffering from skin diseases, injuries or hunger are constantly restless and thus not available for experiments. As a further advantage experimental strategies and mathematical procedures, such as Fourier analysis, so far successfully applied in optomotor studies can also be applied to investigate the electromotor response and thus allow the quantification of the performance of the electric object detection system in terms of phase and gain values as functions of temporal and spatial frequencies.

Considering the sinusoidal movement of the 'Plexiglas' arrangement as input and the sinusoidal motion of the fish as output, one can determine the transfer function of this system by applying different input frequencies and measuring the gain and phase of the fish's response. As Fig. 2*a* shows, the fish maintains a high and constant gain over at least three octaves (0.025 to 0.2 Hz) whereas it follows with decreasing amplitude and increasing lag at oscillation frequencies greater than 0.2 Hz. This deterioration in performance, however, may be a consequence chiefly of the mechanical inertia of the fish rather than an overt sensory deficiency. As high frequencies of oscillation are applied, the fish tries to follow by stronger sideways undulations of its anal fin but never reaches a sufficient speed to follow the 'Plexiglas' strips over their total excursion.

In analogy to visual performances one would expect weaker responses as narrower 'Plexiglas' strips are chosen for the arrangement shown in Fig. 1 and that responses should finally cease as the threshold size of electrically-detectable strips is reached. A fish hovering in the swinging 'Plexiglas' arrangement responds to the reversal of its motion with a certain delay. This leads to a corresponding phase lag between the

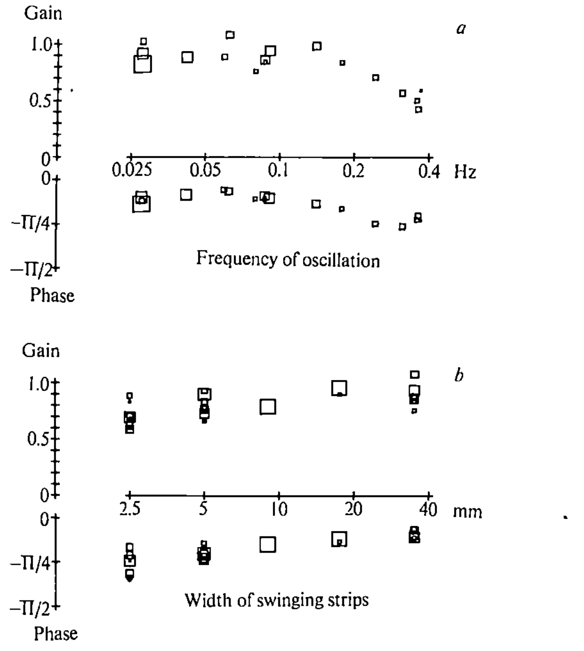

Fig. 2 Gain and phase of electromotor response in one *Eigenmannia* specimen as functions of frequency of oscillation (*a*) and width (*b*) of swinging strips in 'Plexiglas' arrangement shown in Fig. 1. Experimental specifications: *a*, 'Plexiglas' strips 35 mm wide, 1.5 mm thick, peak to peak amplitude of oscillation 20 mm, inner distance between strips 2 and 3 35 mm; *b*, frequency of oscillation 0.1 Hz, peak to peak amplitude of oscillation 15 mm, inner distance between strips 2 and 3 30 mm, all strips 1.5 mm thick. Linear size of squares proportional to length of individual data record available for Fourier analysis, maximum length 120 s.

sinusoidal motion of the fish and the sinusoidal motion of the 'Plexiglas' strips. When narrower strips are chosen the fish's response is more delayed (Fig. 2*b*). Finally, at a strip width of 2.5 mm the lag may become so large that the fish almost collides with a swinging strip before reversing its motion. It thus seems that narrower strips have to approach and to withdraw over greater distances before the fish can decide in which direction it has to travel.

The measurements illustrated here were all obtained from one specimen which gave consistent results during several weeks of experiments and required frame by frame measurements of cine film, followed by semiautomatic digitization and computer calculation of phase and gain. To obtain a first estimate of individual variation four more specimens were tested with 2.5 mm wide strips at an oscillation frequency of 0.1 Hz. The mean phase and gain values found ranged between $-\pi/8$ and $-\pi/4$ and between 0.9 and 1.1 respectively and thus did not differ much from the corresponding values in Fig. 2*b*.

One may argue that a fish following the swinging 'Plexiglas' arrangement only escapes from the strip which approaches its side rather than follows the opposite strip as it moves away. However, when the fish happens to hover a few centimetres outside the arrangement, it still follows both the approach and the withdrawal of the strips, all of which lie to one side of the fish; it follows, to be sure, in a less stationary fashion and may lose "contact" after a few cycles. The 'Plexiglas' arrangement thus operates in a "push-pull" fashion when the fish hovers inside, which seems to account for the greater stability of its response.

Eigenmannia is thus able to control its distance from nearby objects of different conductivity than water. This mechanism should enable the fish to hold a certain position in a habitat of swaying plants without depending on visual or mechanical cues.

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Mercury and Other Metals in British Seals

INCREASING concern about environmental pollution has led to many investigations of heavy metals and their distribution in the sea, air and biological materials. There have, however, been few investigations of seals and here we give some preliminary results of analyses of British seals. Soft-tissue samples were taken from ninety-six seals (Table 1), both common seals (*Phoca vitulina*) and grey seals (*Halichoerus grypus*), from six sites around Britain between February 1968 and January 1972; these were collected during the course of long-term population studies. Results from some areas lack data on samples from the middle age-group as mature seals cannot be aged by external features but by examination of canine teeth[1,2]. Data from East Anglia for both species were pooled because of small sample sizes. Brain and liver samples were obtained from most seals and kidney and teeth from about a third; all tissues were placed individually in uncontaminated polythene bags and stored at −20° C. The wet samples were digested in nitric acid and total mercury was estimated using a Perkin–Elmer 305 atomic absorption spectro-

Table 1 Geographical Distribution and Age Groups* of Seals Sampled

Area	Species	0–0.9	1.0–5.9	6–10.9 yr	11–15.9	16–20.9	21–25.9
1. Wash	*P. vitulina*	9	1	2	1		
2. Scroby Sands	*H. grypus*	2 (3)	1 (4)	(1)	1 (1)		
	P. vitulina	2	3				
3. Farne Isles	*H. grypus*	5	2 (3)	1	2	2	1 (2)
4. West Scotland	*H. grypus*	(3)	(2)	(2)		(1)	
	P. vitulina	4	3	4	2		
5. Outer Hebrides	*H. grypus*	5		1		2	3
6. Orkney and Shetland	*H. grypus*	4 (9)	3 (5)	1		(1)	

* Seals were aged to the nearest whole year, but have been grouped to simplify the Table.

Figures in parentheses indicate additional seals from which tooth samples only were taken.

photometer (AAS). Cadmium in soft tissues was estimated by AAS using a flame technique; teeth, collected from 1968 onwards, were dissolved in nitric acid and analysed on a Perkin-Elmer 303 AAS for lead, cadmium, copper, chromium and zinc.

The results, summarized in Table 2, showed that there were regional variations in mercury concentrations in the brain, Hebridean values being higher than those of the Farnes; the results from the Hebrides and Farne Islands were significantly greater than those from all other regions. These variations may be a consequence in part of the differential passage of mercury compounds from the blood to brain tissue, the "brain-barrier" effect having previously been demonstrated in toxicological studies[3]. Methyl-mercury, which is extremely toxic[4], can easily pass this barrier[4,5] compared to inorganic and most organic mercurials.

Except for the common seals from West Scotland, the concentrations of mercury in the brain did not increase with age (Fig. 1), suggesting that a ceiling level is reached early in life. Mercury concentrations in liver were positively correlated with age (Fig. 2) in five of the six areas, the exceptions being Shetland seals in which levels were lower than those from all other areas. Analysis of combined data from all areas gave a correlation coefficient of 0.78 ($P<0.001$). Analysis of variance on the transformed data for all areas showed that there was an overall significant difference between some of the slopes ($F=2.979$, $P<0.05$), due to the East Anglia and West Scotland regressions having greater slopes (that is, higher build-up of mercury in liver) than the other three areas. Although *P.*

Table 2 Mercury and Cadmium Concentrations in British Seals (1971–1972)

Areas 1 and 2 East Anglia			Area 3 Farne Islands			Area 4 West Scotland		Area 5 Outer Hebrides			Area 6 Shetland		
Hg brain	Hg liver	Hg kidney	Hg brain	Hg liver	Cd kidney	Hg brain	Hg liver	Hg brain	Hg liver	Cd kidney	Hg brain	Hg liver	Cd kidney
0.4	29.9	13.7	0.7	84.2	5.3	0.4	30.1	0.7	113.0	11.6	0.3	4.9	2.2
±0.2	±38.7	±0.2	±0.2	±68.9	±4.0	±0.1	±18.2	±0.2	±68.0	±7.5	±0.1	±3.6	±2.0
(13)	(15)	(2)	(7)	(7)	(5)	(8)	(9)	(6)	(6)	(4)	(8)	(8)	(8)

Mean values ±SD in p.p.m. wet weight.

Figures in parentheses are the number of samples. Cadmium values for all brain and liver samples were less than 1 p.p.m. Pup and foetus values (involving areas 3, 4 and 5 only) have been omitted from the calculations, as they represent maternal pollutants; a mother and her pup/foetus can be considered as one and the same marine-feeding unit.

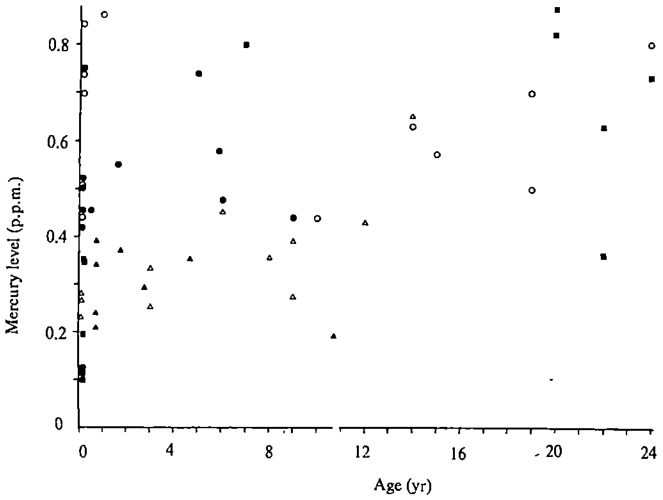

Fig. 1 Mercury in seal brain 1971–72 (p.p.m. wet weight total mercury). Correlation of concentration with age: ●, East Anglia, $r=0.459$, $P>0.1$; ○, Farne Is., $r=-0.068$, $P>0.1$; ■, O. Hebrides, $r=0.545$, $P>0.05$; △, West Scotland, $r=0.778$, $P<0.01$; ▲, Shetland, $r=-0.537$, $P>0.1$.

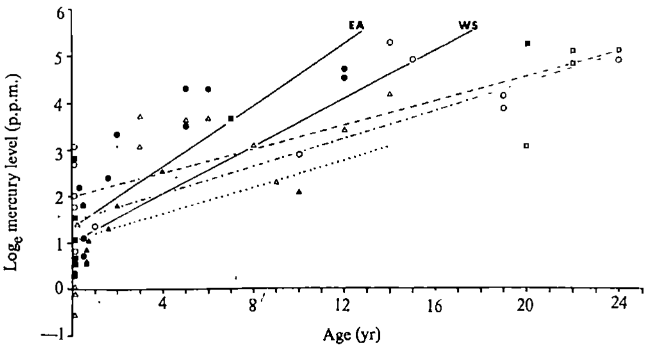

Fig. 2 Mercury in seal liver 1971–72 (p.p.m. wet weight total mercury). ——●, East Anglia (EA), regression line: $y=0.340x+1.282$, correlation with age: $r=0.845$, $P<0.001$; --- ○, Farne Islands, $y=0.132x+2.021$, $r=0.832$, $P<0.001$; ·—·—· ■, Outer Hebrides, $y=0.150x+1.467$, $r=0.886$, $P<0.001$; ——△, West Scotland (WS), $y=0.254x+1.036$, $r=0.726$, $P<0.01$; ... ▲, Shetland, $y=0.146x+0.993$, $r=0.689$, $P>0.05$.

vitulina constituted 88% of the seals from the East Anglia/West Scotland group, and seals from all other areas were *H. grypus*, any conclusions about differences between the species must remain tentative at present, as the age spectra of each group differed, and there were more older seals in the samples of *H. grypus*. Further extensive sampling is needed to establish if the straight line model for Log$_e$ of mercury accumulation with age is adequate.

Table 3 Grey Seal *Halichoerus grypus*—Pup/Mother Ratios of Mercury and Cadmium Residues (1971–1972)

Area	Amount of mercury in brain	Amount of mercury in liver	Amount of cadmium in kidney
3. Farne Islands	1.02 (5)	0.12 (5)	0.08 (3)
5. Outer Hebrides	0.57 (5)	0.06 (5)	0.05 (3)

Mean values p.p.m. net weight.

Number of observations (pup/mother pairs) are shown in parentheses.

Cadmium was found in comparatively large quantities (up to 22 p.p.m. wet weight) in all kidneys (Table 2), the concentration tending to increase with age ($P<0.001$). Brains and livers, however, contained less than 1 p.p.m.

Mercury and cadmium in pup tissues varied in relation to maternal levels (Table 3) and in general reflected the tendency for some metals to show an age accumulation in certain tissues. Other studies have shown that the placenta constitutes a barrier to the passage of most mercury compounds except methyl mercury[6] which passes into the foetus easily and may reach a concentration comparable with that in the mother[7].

Table 4 Metal Concentrations in Grey Seal Teeth

Area	*n*	Lead	Zinc	Chromium	Copper	Cadmium
1. 2. East Anglia	20	36.7± 7.5	103.9±56.4	19.9±7.9	4.9±6.2	3.9±0.8
3. Farne Islands	15	30.6± 7.7	142.4±63.6	14.7±6.5	6.9±5.3	3.5±0.5
4. Outer Hebrides 5. Inner Hebrides (West Scot.)	13	32.7±10.5	145.8±66.9	14.3±3.9	4.1±1.8	3.8±0.7
6. Orkney and Shetland	15	37.0±10.4	85.2±45.3	18.1±8.4	4.7±4.7	5.2±2.5
Correlation with age of seal	63	$P<0.001$ (−ve)	$P<0.001$ (+ve)	$P<0.001$ (−ve)	$P>0.1$	$P<0.05$ (−ve)

Mean ± SD p.p.m. dry weight[11].

Lead, zinc and chromium were found in molar teeth, with cadmium and copper in smaller quantities (Table 4). Analysis of the data in relation to age showed a progressive decrease in concentrations of lead, cadmium and chromium; young seals contained higher lead levels than older individuals ($P<0.001$), the chief change occurring at about 5–7 yr of age. This may reflect the fact that, initially, enamel, cement and dentine are laid down together in molar teeth, but after about 5 yr only small amounts of biologically inert cement are deposited[2], so that relatively little extra material is incorporated into the tooth. Metal concentrations will tend to remain stable after 5 yr or decrease slightly with the slow accretion of cement and may therefore reflect, to some extent, the metal concentrations encountered during the first 4 or 5 yr of life. Zinc levels, however, showed a reverse trend, increasing with age. Regional comparisons of metals in teeth revealed no clear pattern, but cadmium and lead decreased in the order Orkney and Shetland, East Anglia, Hebrides, Farne Islands. There was no significant difference between lead residues from Scotland and East Anglia.

Our preliminary study suggests the following: 1, Mercury reaches a ceiling level in brains at about 12–18 months; residues in man reach similar levels and also show no correlation with age[5,8]. 2, Mercury tends to accumulate in the liver with age, reaching fairly high levels. 3, Cadmium accumulates in the kidney, as it does in man[9], where it is known to occur in higher concentrations than in other organs[10]. 4, There do not seem to be regional differences in metal concentrations in teeth, but some metals were found in greater quantities in younger seals than in old ones, probably related to the pattern of tooth growth. 5, There are regional differences in the amounts of mercury in the tissues, but there does not seem to be any relationship with possible agricultural or industrial pollution.

Our results demonstrate the importance of taking age into account when studying the amounts of metals in biological tissues. Conclusions based on small samples, when the age of the animal is unknown, can be misleading.

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Egg Taints: Association with Trimethylamine

MILLER[1] has described brown-shelled eggs with a characteristic taint produced in various parts of the United Kingdom during autumn 1971 and early 1972; this problem has occurred extensively in the UK over the same period in earlier years but it was much more apparent in 1971–1972.

Brown-shelled eggs from the eight commercial flocks of birds which we have investigated contained from 6% to 27% of eggs described as "fishy" or "crabby", similar to those of Miller[1]. No tainted white shelled eggs have been reported to us.

Birds from a typical large outbreak were examined and 20 birds of a single strain with "bad breath" were selected since this condition had been associated with tainted eggs[1,2]. Using the identical diet and individual caging the eggs produced were examined individually and those regarded as significantly tainted by all the testing panel (3 or 4 subjects) were used for investigation of the compound responsible for the taint. Tainted eggs (1,400 g, *p*H 7–8) were freeze dried and the distillate (1,100 ml.) collected at −198° C. The condensate was melted, acidified (*p*H 3) and freeze dried to give crude amine hydrochlorides (60 mg); gas chromatography on an alkaline support[3] showed that the characteristic odour was coincident with the peak corresponding to trimethylamine and this identification was confirmed by mass spectrometry. Tainted eggs, including examples from Miller's experiments[1], contained more than 1.0 $\mu g\ g^{-1}$ of trimethylamine whereas untainted eggs contained less than 0.1 $\mu g\ g^{-1}$; most of the trimethylamine $(\sim 95\%)$ was present in the yolk. The odour threshold concentration which we have determined for trimethylamine in water (0.0005 $\mu g\ g^{-1}$) is below that in tainted eggs.

On our own control diet these selected birds produced no tainted eggs; when trimethylamine hydrochloride (100 mg day^{-1}) was fed to nine of the twenty birds, they all produced characteristically tainted eggs containing trimethylamine (3 to 40 $\mu g\ g^{-1}$) within six days.

Repetition with fifteen 52-week-old brown birds with no record of producing tainted eggs, showed, however, that only two of these birds produced eggs containing trimethylamine (10 to 40 μg g^{-1}).

The production of tainted eggs is therefore, like bad breath[1,2], associated only with certain individual birds. Gas chromatography/mass spectrometry on samples of breath from the birds described by Miller[1] has shown that the major odorous constituent is dimethyl sulphide and aqueous solutions of dimethyl sulphide at concentrations of 10^{-14} to 10^{-15} g $ml.^{-1}$ have odour characteristics extremely similar to those of "bad breath". We have not, however, been able to confirm a direct relationship between these two effects.

These preliminary results indicate that a complex situation is involved. A dietary source of trimethylamine or of a trimethylamine precursor is necessary and we have been able to reproduce the phenomenon in selected birds by feeding a diet containing particular batches of rape seed meal. This meal contained less than 1 μg g^{-1} of free trimethylamine, and is not therefore an immediate source of preformed trimethylamine, which must arise by an, as yet, undefined route. The coincidence of at least two factors, in the bird and in the diet, for the production of this situation is clearly necessary and is probably the origin of the sporadic nature of the problem. There is no evidence that fish meal is involved. Further investigations are in progress.

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Temperature Coefficient (Q_{10}), Seed Germination and Other Biological Processes

A COMMON method of comparing rates of reactions or processes in biological systems is the use of the temperature coefficient, or Q_{10}, the ratio of the rates of a reaction or process at $(T+10)°$ C and $T°$ C. James[1] says of the Q_{10}: "As it is readily calculated, easily understood, and carries no theoretical implications, it remains a useful and favourite method of recording the temperature relations of complex biological processes". But because Q_{10} values of different processes tend to fall within certain limits, they are often used to indicate types of reactions that might be occurring.

The basis for this depends upon the description of biological processes in terms of the Arrhenius law[2,3]. This predicts that the Q_{10} values of many chemical reactions obeying the law will be within the range 2 to 3 at room temperature (justifying the van t'Hoff rule) and that Q_{10} values will fall gradually with increasing temperature. Several biological processes, whether seemingly governed by individual chemical reactions or whether describing gross physiological processes of tissues or populations, do seem to obey this law[2,4], but in many biological processes it is normal to find that Q_{10} values fall with increasing temperatures at a rate faster than would be predicted from the Arrhenius equation[1,2].

One approach to this discrepancy between chemical and biological kinetics is to consider Q_{10} in relation to the total temperature range of the process. In chemical kinetics the relative constancy of Q_{10} for reactions following the Arrhenius law is dependent on the slow rate of change of the product of T and $(T+10)$ K. In a physiological process there is a temperature range which is comparatively narrow and beyond which the process or reaction does not occur. The Q_{10} is therefore calculated for a process that rises from zero to a maximum and then falls again to zero within a temperature range probably not exceeding 40 to 50° C. Often consideration of Q_{10} is restricted to the increasing phase only. Any Q_{10} for a biological process calculated with the minimum temperature as the lower temperature limit (when the rate is just not detectable) has a value of infinity; any Q_{10} calculated with the maximum temperature as the upper temperature limit has a value of zero. Under normal circumstances, and over the whole temperature range in which the process occurs, the value of Q_{10} for that process must fall from infinity to zero with increasing temperature. During the phase of increasing rate of reaction, when it could be argued that the effects of enzyme inactivation due to low or high temperature are possibly not acting, there may be a range of temperature over which Q_{10} is relatively stable, but this is not necessarily the case.

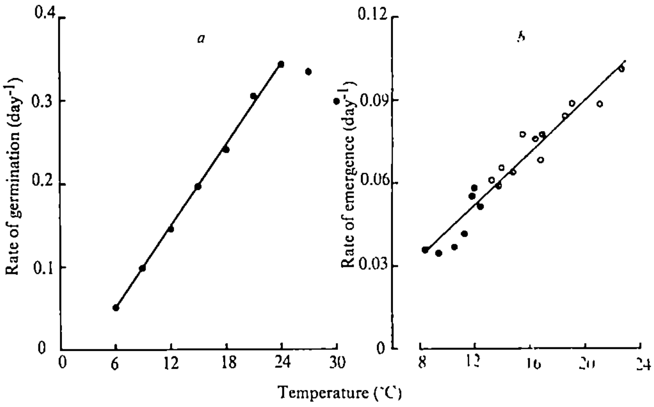

Fig. 1 *a*, Rate of germination against temperature for carrots in the laboratory; *b*, rate of emergence against mean soil temperature for carrots in the field.

Exceptions are to be found, for instance, in values of Q_{10} derived from germination data. Kotowski investigated the rates of emergence from sand of several vegetable species[5] and found that Q_{10} values were higher at lower temperatures and lower at higher temperatures with mean values usually in the range 2 to 3.5. He concluded that the time rate of the production of seedlings at different temperatures agreed well with the van t'Hoff temperature coefficient ranging from 2 to 3. But the Q_{10} values given by Kotowski for all species were as low as 1 to 1.5 between 25 and 30° C and as high as 5 to 8 between 8 to 11° C and 4 to 8° C.

Harrington[6] followed the germination on paper of a range of vegetables and Leach[7] the emergence from sterile soil of five crop types. Commenting on the results Harrington stated: "Kotowski found Q_{10}s of 4 to 12 between 4 and 8° C; Leach's results are comparable when the Q_{10} is calculated from his data and the results of this (Harrington's) research are similar, averaging 11.26 between 0 and 5° C. The Q_{10}s steadily decrease to an average Q_{10} of 0.84 between 30 and 35° C. This shows how extremely temperature dependent are the reactions involved in germinating seeds."

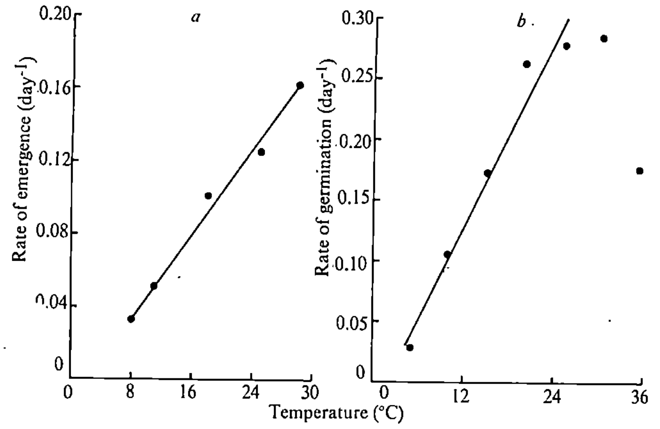

Fig. 2 Rates of emergence and germination of carrots at constant temperature. *a*, Emergence from sand[6]; *b*, germination on paper[6].

Recent work[8] has shown that for carrot seeds in a temperature range of almost 20° C the relationship between rate of germination and temperature in the laboratory, and between rate of emergence and mean soil temperature in the field, is linear to a good approximation. The data (Fig. 1) were obtained in 1972, but allowing for problems associated with extreme moisture stress similar results have been obtained using a number of different carrot seed lots in previous years. Similarity of the Q_{10} values calculated for different temperature intervals with those of Kotowski[5] and Harrington[6] suggests that their results might also show linear relationships if plotted in this way, and in spite of the small number of points on the graphs, this did seem to be the case for a restricted temperature range (Fig. 2). Plots of rate of germination or emergence against temperature for a limited number of other species have also given good approximations to linear relationships (Fig. 3*a*, *b*, *c*). But in some cases where there seems to be a good linear relationship (Fig. 3*d*) an analysis of variance shows that there are significant quadratic and cubic components in the variation due to temperature, indicating that the curve is probably sigmoid over this temperature range.

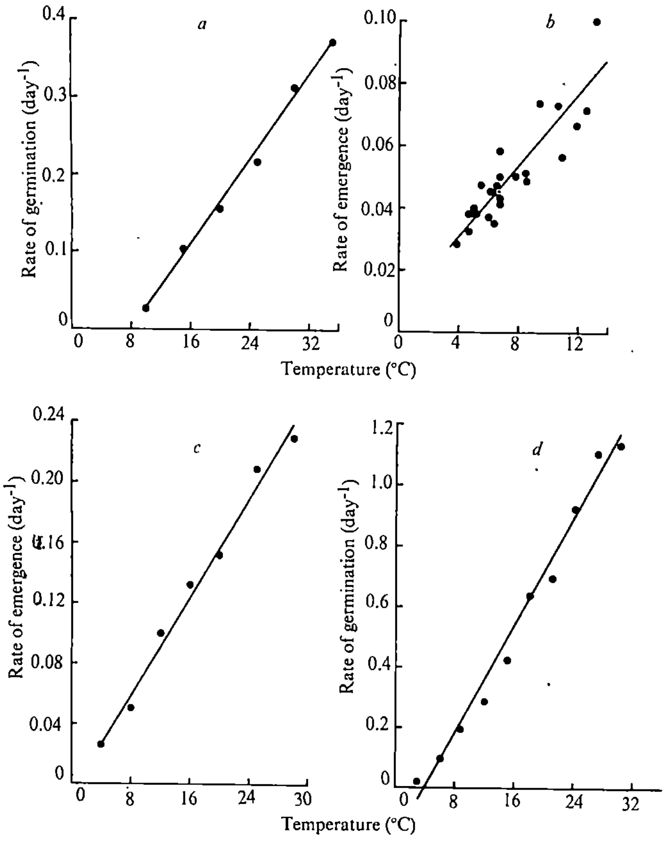

Fig. 3 Rates of emergence or germination against temperature or mean soil temperature for: *a*, snap bean on paper[6]; *b*, spring oats in soil (based on the time to appearance of the crop)[10]; *c*, wheat from sterile soil[7]; *d*, cauliflower on paper.

The occurrence of a linear relationship does not necessarily suggest that there is a single dominant rate-limiting process governing germination that is itself linearly related to temperature. It is probable that the rate of a biological process is determined by a complex of reactions and processes each independently affected by temperature[4], and a more likely explanation of the relationships is that in some cases the rate-limiting reactions involved combine to give, fortuitously, a linear dependence of rate on temperature over a relatively wide temperature range.

Whatever the underlying principles behind the linear relationship may be, these relationships have considerable potential use for the development and justification of cropping programme models based on heat unit or linear temperature/growth concepts[9] and provide a convenient means for comparing the germination rates of different seed lots (Fig. 4).

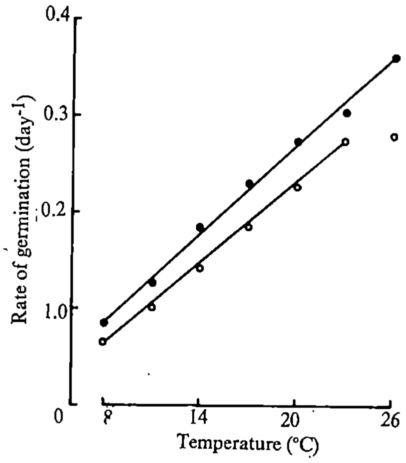

Fig. 4 Rate of germination against temperature for two carrot seed lots. The difference between the slopes was significant ($P<0.05$).

Concentration on the Q_{10} as an easy and convenient means of expressing such relations can be misleading and, perhaps more important, can obscure simple and potentially useful relationships.

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BOOK REVIEWS

Machines of the Past

The Computer from Pascal to von Neumann. By Herman H. Goldstine. Pp. x+378. (University of Princeton: Princeton, New Jersey, January 1973.) £6.25.

It is rare to be able to say of a new book that it is the first in its field, but such is the case with this production of Herman Goldstine. There has, up to now, been no volume devoted exclusively to the history of computers and computing, although of course many books published in the last twenty years have had a preliminary section dealing with matters historical.

Dr Goldstine has a unique title to write a history of computers because he was one of the people involved in the earliest days of the electronic era. Furthermore, as practically the last representative of the famous Princeton Institute for Advanced Studies group, Goldstine is performing a service in establishing precisely the role which the Institute played in the development of electronic computation. Goldstine himself seems to have doubts whether the Institute's role has been sufficiently recognized. From a now completely impartial position within the field, I find this one of the few statements I would dispute. Wherever electronic computation is talked about, and wherever expert groups gather, the Institute is usually credited, even now, with pioneer efforts in the field of true electronic computation.

Trained originally as a mathematician and physicist, Goldstine worked with the Moore School group on the ENIAC and its successors, transferred to the Institute for Advanced Study, where he worked with the late John von Neumann, succeeded von Neumann as Project Director, and then, with the closure of the Institute's computer project, moved to industry, which he considers the most active area now. He has held distinguished posts, mostly with the IBM Corporation, where he was Director of Mathematical Science at the Thomas J. Watson Research Centre, Director of Scientific Development of the Data Processing Division, and is now enjoying an honoured position as an IBM Fellow.

The book has three main parts—the first effectively historical, the second concerned with the great upsurge of computer activity in World War II, and the last dealing with activities at the Institute for Advanced Study after the conclusion of hostilities. There is also an Appendix. The opening chapter on the historical background is, perhaps, slightly skimpy, although the author has made an original contribution in his discovery of the work of Wilhelm Schickard who designed and built in 1623 a machine capable of automatic addition and subtraction and semi-automatic multiplication and division. Unfortunately, Schickard's actual device is lost, although some indications of its method of operation still exist, and the IBM Corporation, as part of its general historical and cultural activities, has made a version of the machine which is illustrated in the book. Schickard clearly anticipated the work of Blaise Pascal who is usually credited with the invention of the first adder-subtracter in 1642. Leibniz, whose multiplier dates from about 1690, was also anticipated, although it would seem that Schickard's methods of semi-automatic multiplication and division were quite different from the step wheel conceived by Leibniz.

As usual in books on computation, the author of this one spends some time on the English genius, Charles Babbage. Dr Goldstine appears to take a position which has been espoused by several recent writers that Babbage was a misanthrope and a crank. Here I disagree with the author, who has not, perhaps, read some of Babbage's works on the nature of industry in depth. These show that, far from being a man who continually devised new solutions to computational problems and abandoned his partially constructed devices without completion, he devoted much of his energy to the improvement of manufacturing techniques and technology in England. It is true that the technology of the time was not adequate to realize Babbage's conception, but, far from "ending it all with a bare bodkin", Babbage "took arms against a sea of troubles", and if he did not end them, he at least made some impact on the scene.

These are minor criticisms, however, for the book goes on to a fascinating discussion of the origin and provenance of the ENIAC, and includes many sidelights, generally unknown, on the wrangling and disharmony which eventually disrupted the Moore School team. The discussions about the work at the Institute for Advanced Study are, of course, seminal, and, apart from brief mentions in obituary notices of John von Neumann, have not hitherto been revealed. They are an important contribution to the history of computation. Perhaps inevitably, portions of the book, and particularly the Appendix, which deal with developments outside of the United States, are less satisfactory than the rest. For example, the attribution to Atanasoff on page 123 of the development of binary methods in digital computation completely ignores the claims of E. Williams Phillips, the English actuary whose contributions are well documented. Yet again, perhaps too much importance is attached to certain evanescent developments such as the Forrester-Williams storage tube. Perhaps more important is the omission of reference to the work of K. D. Tocher, the actual inventor of micro-programming despite the urgent protestations of some later rediscoverors. These things, however, are minor blemishes.

The book is first-rate: it is written in a style that all can understand, and where advanced mathematical techniques are mentioned, sufficient background is provided for even the lay reader to understand what is going on. Goldstine has a lucid style, and it is greatly to be hoped that in due course we shall see other productions from his pen.

Andrew D. Booth

Observations on the Sun

Solar Activity: Observations and Predictions. Edited by Patrick S. McIntosh and Murray Dryer. Pp. xv+444. (MIT: Cambridge, Massachusetts and London, November 1972.) $17.50.

With this publication the MIT series on *Progress in Astronautics and Aeronautics* reaches its thirtieth volume. Arising out of a 1970 conference held in Huntsville, Alabama, the book comprises twenty-five papers reviewing current knowledge of solar activity, its effects on the interplanetary and terrestrial environments, and the possibilities of predicting such phenomena. Compared with the material found in some such conference products, this work effectively avoids the pitfalls of over-specialization and subjective research opinions, a success perhaps attributable to the non-specialist format requested from contributors and to the inclusion of extra papers to fill gaps in the conference material.

The papers fall into four sections, starting with "Solar Observations and Theories", moving out through the "Interplanetary Medium" to consideration of "Geophysical Responses to Solar Activity" and completed by studies of "Progress in Forecasting Technology". J. W. Evans leads off with his "Introductory Review of Solar Activity", a highly competent and concise account of solar phenomena and nomenclature for the newcomer to the field, spoilt only by some lack of attention to numerical detail and descriptive clarity in places. Further papers in section 1 divide equally between observations of large and small scale solar magnetic field distributions and of flare particle, radio, ultraviolet and X-ray-line emissions, rounded off by Sturrock's résumé of magnetic flare models. Chief omissions are adequate discussion of hard X-ray observations (an important flare diagnostic) and explicit description of optical flare characteristics other than inference of flare fields from Hα filtergrams.

Continuing through the papers on interplanetary and geophysical aspects, the reader encounters a continuing high quality and density of information. Particularly valuable to the newcomer are the reviews of the solar wind by Dryer and Cuperman and of magnetospheric particles by Paulikas. Despite all this material, however, a shadow of the stamp-collecting era remains looming over the science of solar activity. Application of games theory (Smith) and of Markov chains (Mogilevsky) gives "successful" activity predictions for only a few days, little better than suitably "accurate" and "skilful" subjective classification of active regions. (Predictions of heliographic locations of activity are more satisfactory.)

Thus, barring a spectacular improvement in these techniques or a conceptual breakthrough in activity theories, McIntosh and Dryer's editorial anticipation of the military, astronautic and ecological benefits to accrue from activity predictions must be, at best, long-term ideals. Viewed, however, as a unifying account of solar activity, their book can be recommended to all.

John C. Brown

Critique in Palaeontology

Models in Paleobiology. By Thomas J. M. Schopf. Pp. vi+250. (Freeman, Cooper: San Francisco, 1972.) $9.50.

At last we have been provided with a thought-provoking text on invertebrate palaeontology. Tom Schopf is to be congratulated not only on having the vision and temerity to question the motivation and direction of palaeontological enquiry but also for producing this timely panoramic critique at a comparatively moderate price.

The volume comprises ten essays on various aspects of palaeontological analysis and synthesis grouped under the headings of (1) "Models in Paleobiology", (2) "Morphology", (3) "Population and Evolution", and (4) "Distribution". They result from a 1971 symposium on models in palaeobiology jointly sponsored by the Paleontological Society and Geological Society of America at their annual general meeting in Washington DC. The styles of the presentations vary from author to author but each is introduced by a pertinent précis by Schopf. Thus no matter how complex and abstruse some of the articles may at first seem, Schopf has succeeded in ensuring that all contributions are intelligible even to those geologists who suffer from minimal and/or inappropriate scientific backgrounds. This is no mean feat since the discussions liberally impinge on the cognate sciences of evolutionary palaeontography, biogeochemistry and statistics.

Schopf's implied secondary theme that all too frequently the last decade has witnessed an explosion in unquestioning and uncanalized palaeontography is hard to refute. In assembling these papers he has effected a heroic job which ought to revitalize the science and its utility. Even now there are deficiencies which are a salutary reflexion on the inadequacies and inappropriateness of our scientific trainings. Too often we are trained to see the trees at the expense of seeing the wood. The advances quoted in this volume are frequently simple and derive from using the methodology of a cognate science rather than a large body of complementary subject matter. The basic deficiency underlying all the themes is our bland neglect of the physiological parameters which have a profound influence on both biogeochemistry and ecology, which thereby affect evolutionary palaeontography and culminate in the difficulties which arise in statistical analysis as a result of cumulative omission. This does not mean that statistics should be ignored as unsound, but that it should be treated with the reverent constructive caution so admirably displayed by Simberlof. For, as Eldredge and Gould so rightly, but damningly, imply, we tend to report those things for which we have explanations while ignoring those aspects which embarrass us. This is perhaps epitomized in Dodd and Schopf's truism that the biogeochemical data available to us are woefully inadequate as the geochemical results have seldom tested the intimate relationships which would have been required had the enquiries rested on sound biological principles. This can easily be seen to result from the fact that all too frequently undergraduate geologists align themselves with either the physical sciences or the biological sciences but seldom both. Thus, quite apart from the intrinsic value of the variety of approaches clearly analysed, the volume makes salutary reading for those concerned with both educational and applied aspects of geology and the earth sciences.

This book should be compulsory reading for all geologists and earth scientists who direct departments and assess research proposals. It will provide palaeontologists with many happy hours of brain-teasing, stimulation and self-assessment. It should intrigue the curious undergraduate, absorb the graduate student, and infuse panic in the hearts of those palaeontographers who have got stuck in a rut. No geology department library can afford to be without this volume, for its intellectual applications are not limited to palaeontology alone. This tome has achieved for palaeontology an analogy of what the Geophysical Laboratory, Washington DC, contributed to petrology some decades ago.

J. A. E. B. Hubbard

Vibrations in Fluid

Sound, Structures and Their Interaction. By Miguel C. Junger and David Feit. Pp. x+470. (The MIT: Cambridge, Massachusetts and London, October 1972.) $27.50.

There are many books on acoustics and many on vibrations. Some even consider the radiation of sound from vibrating surfaces and others the vibration induced in a structure by an impinging sound wave. There is, however, no book devoted to the interaction between structures and the surrounding fluid. At best a chapter or two in existing texts might be devoted to particular aspects of the interaction. The majority of written work on this topic is spread through the technical literature, and not a small portion of this is classified because of its underwater naval applications. Dr Junger himself has been responsible for several significant developments in underwater acoustics.

This textbook is the first book to draw together much of this material and to present it in a coherent way. The book arose from the need, at postgraduate level, of a text on the interaction between a vibrating structure and the surrounding medium. The interaction becomes most important with dense fluids such as water, but there are cases in which even the weaker coupling with air is important. The results will also be important for containment structures such as gas cooled nuclear reactor circuits. The book is largely theoretical but does cover cases and conditions which are of interest in practical problems. Many valuable results which are scattered through the literature are brought together in this book. Naturally these include Dr Junger's own contributions—for example his classic work on the radiation of sound from cylindrical shells vibrating in a dense fluid is well presented. This is of value from the point of view of far field radiation but also from the feedback effect giving the damping of the vibrating structure.

The basic acoustic theory of sound sources is clearly presented and developed to multiple arrays and radiation from vibrating surfaces. The authors then go on to consider radiation from vibrating surfaces submerged in a fluid and complete this with a comprehensive treatment of cylindrical and spherical shells. The basic theory of the scattering of sound by rigid bodies is followed by a chapter dealing with elastic scatterers and reflectors. This latter topic is of growing importance and has not been treated in the standard textbooks. The approach throughout is deterministic but asymptotic techniques for high frequency acoustical, vibration and interaction problems are presented.

There is no mention of the growing amount of work on the statistical energy method of analysing structuro-acoustic interaction problems. Perhaps this could form a second book in the series on structural–acoustic coupling.

In conclusion I consider that this book fills the need for a textbook which presents in a comprehensive way all the acoustic and vibration theory which is required to arrive at a deterministic type of solution to interaction problems. It will be of great value to postgraduate students and research and development workers in this field of growing interest.

B. L. CLARKSON

Mercury Poisoning

Mercury in the Environment: an Epidemiological and Toxicological Appraisal. Edited by Lars Friberg and Jaroslav Vostal. Pp. 215. (CRC: Cleveland, Ohio, 1972.)

GOING along with the current advancement in chemical industry, the water pollution caused by mercury is gradually increasing. Since the human hazard of mercury, well known by the name "Minamata disease", exists in Japan where the first mysterious poisoning occurred, this problem has been taken up as an urgent matter at an early stage, more so than in other parts of the world.

While this is becoming an increasing worldwide problem, the book *Mercury in the Environment* is timely and the contents useful.

This book first introduces the up-to-date methods of analysis of mercury, both organic and inorganic, and also introduces transport and transformation of mercury in nature, and possible routes of its exposure. Secondly, it discusses metabolism and especially about distribution, retention and excretion in the living body, as well as including the latest information about symptoms and signs of the poisoned individual. To discover the normal content of mercury in tissues, urine and blood of the body, the toxic effect of mercury compounds, how they are formed, and the amounts which cause intoxication are being minutely investigated. Lastly, they comment on the genetic effects of mercury.

Gathering all possible literature concerning environmental pollution and damage caused by mercury, this book comprehensively covers the current information and knowledge of the mercury problem.

TADAO TAKEUCHI

Electromagnetic Waves

Theory of Ionospheric Waves. By K. C. Yeh and C. G. Lui. Pp. xiv+464. (Academic: New York and London, December 1972.) $24.

THIS is essentially a monograph concerned with the propagation of electromagnetic waves in an ionized gas, featuring those aspects of propagation that are of most concern to the radio physicist. Thus the medium is treated almost exclusively by means of its fluid approximation and equivalent dielectric characteristics, and propagation is treated by means of an inevitable succession of stages of increasing complexity: without and with a background magnetic field, without and with a significant temperature of the plasma, without spatial variations and then with gradual variations and random, scattering variations, of background parameters.

Most of the topics treated cannot help but be familiar to readers of other texts on ionospheric radio propagation or on waves in plasmas. What will make this text distinctive and perhaps preferable for some such readers is its own particular choice of path through well-trodden territory. An outline of the ionosphere is given for initial stage-setting, along with a forewarning of the dyadic notation that permeates later pages. A purported review of electromagnetic theory is hardly what it calls itself, but is in fact a much needed summary of concepts and techniques applicable to the excitation and propagation of electromagnetic energy in dielectric media. Two appendices to the volume, concerned with the method of steepest descents and radiation fields from localized sources, might well have formed an integral part of this review. Plasma properties are then introduced, first via such processes as screening and longitudinal waves, and only later through the more usual route, at least for radio physicists, of the general spectrum of waves with its various special cases: high frequencies, electron and ion whistlers, hydromagnetic frequencies, and so on. The usual geometric optics, WKB approximation, coupled equations, and scattering approximations are followed by a helpful discussion of nonlinear wave processes, a topic too often avoided.

The closing chapter of the volume treats atmospheric waves (except for tides) at ionospheric heights. While it is good for its length, it seems to be more of an afterthought than a correspondingly rounded discussion: a condescension to the book's title, perhaps, or to its inclusion in a series of geophysical monographs. Still, those who buy the volume for its electromagnetic content will find themselves exposed in this chapter to some of the geophysical content of the ionospheric regions that attract their attention, and exposed also to the mathematical linkages—if not to the underlying physical linkages—that unite this geophysical content to the electromagnetic content. It will be a useful exposure for many, and will set the book apart from others of its ilk.

C. O. HINES

The Botanic Tongue

Botanical Latin: History, Grammar, Syntax, Terminology and Vocabulary. By William T. Stearn. Pp. xiv+566. (David and Charles: Newton Abbot, February 1973.) £6.50.

IT is good to see that *Botanical Latin* (more usually known as "Stearn") is again available. Comparison of the first and second editions shows that the pagination is unaltered and the number of additions and corrections small. Unfortunately the second edition has been printed on thicker paper than the first edition and this makes it both heavier and less convenient to handle; but this is a minor drawback.

Anyone who has to read or write botanical Latin or needs to know the precise meaning of a host of technical descriptive terms will find this book invaluable. The introductory fifty-six pages can be read with pleasure and profit by any botanist; these are followed by a section dealing with grammar, hardly light reading but essential to the average taxonomist. The third section, "Syntax and other matters", contains much of general interest, including an extensive list of place names and colour terms. The main part of this section is devoted to examples of diagnoses and descriptions of organisms ranging from algae and fungi to flowering plants. These provide for almost any imaginable situation which might face the describer of a new species when trying to comply with the requirements of the International Code of Botanical Nomenclature for a Latin diagnosis. It also exemplifies the use of Latin as a living modern language devoid of nationalism. Some of its more recent developments are to be seen in the chapter on chemical reactions and tests, though many of the older chemical terms are, of course, derived from pharmaceutical works. The vocabulary of 171 pages, which forms the bulk of the concluding section, is full of information not readily obtainable elsewhere.

As one whose formal training in Latin consisted of six months dreary grind which culminated in passing "Little-go" at the second attempt, I find Dr Stearn's book invaluable. I notice also that some of my colleagues in the Classics department dip into it with interest, and one of them has remarked that if the Romans had sent telegrams they would have developed this sort of Latin. This, I think, provides an objective estimate of the conciseness and clarity which botanical Latin has attained.

It is pleasing that in the seven years that have elapsed between the first and second editions the price has only risen from £5.25 to £6.50. This is a small price to pay for a concise history of systematic botany and of the development of the Latin alphabet and much else besides. T. G. TUTIN

Interpreting Fossils

The Meaning of Fossils: Episodes in the History of Palaeontology. By Martin J. S. Rudwick. Pp. 287. (Macdonald: London; American Elsevier: New York, 1972.) £6.

THERE are probably some university departments of geology in Britain which even now do not offer their students any formal course of instruction in the history of the subject. Too often in the past it has been thought sufficient that the lecturer in palaeontology, stratigraphy, petrology or what you will, should preface his first-year course with a statement on the development of knowledge in that branch or at best devote his introductory lecture to a review of progress of ideas in it over the past hundred years or so. It is often doubted whether students are at that early stage equipped with sufficient basic knowledge of geological terminology to appreciate the analysis which is being made. The dilemma remains that at later stages of the undergraduate course, there is increasing pressure on curriculum time and such addition as the history of the subject is hardly likely to receive any priority.

Dr Rudwick states that his book had its origins in his course of lectures at Cambridge arranged primarily for undergraduates reading history and philosophy of science, but also attended by geology students. The subtitle of the work, rather than its title, indicates its scope and five "episodes" in the history of palaeontology have been chosen for exposition spanning the period from the sixteenth to the mid-nineteenth century.

Beginning with Conrad Gesner's contribution "On Fossil Objects" (1565), Dr Rudwick first develops the theme of the progression from the classification of "fossils" to the first glimmerings of an interpretation of the forms resembling living organisms, and in doing so he introduces the work of a number of the naturalists of Western Europe and relates it to the contemporary philosophical climate of the Renaissance period.

Each of the following chapters raises one or more of the problems which confronted the naturalist in his search for the nature and significance of fossils through the following centuries. The seventeenth century with its tentative approach to the organic theory and the problems arising from the position and altitude at which fossils are found provides the link with ideas on stratification. There follows the period with the constraint imposed by the theologically based limit on geological time, and later an exposition of Charles Lyell's ideas of what the author describes as a "steady-state" model of Earth-history.

The detailed work of the comparative anatomists at the beginning of the nineteenth century is emphasized in reference to their contribution to the various evolutionary theories which followed. The limitations of the palaeontological record as a means of confirmation of the Darwinian theory almost form the climax. Alongside this is the account of the stratigraphical search back into the older rocks for early forms of fossils and for evidence of some beginning of life itself.

Dr Rudwick has been very successful in conveying to the reader the manner in which successive problems in palaeontology were conditioned by the general thinking of the period. To be fully understood, the plausibility of each theory must be judged only in the light of the whole background of knowledge and philosophical temper of the time at which it was propounded.

Writing for two groups of students who have approached the subject from very different directions has imposed special constraints on the author. He has met the challenge with a concise and lucid account which is well illustrated. There is a glossary of about a hundred geological and palaeontological terms; but it has been assumed that the geological student has ready access to a dictionary for definition of such adjectives as teleological, anti-heuristic, and so on.

By reading this book the student of geology, whether specializing in palaeontology or not, could well develop a permanent interest in the broader historical aspects of the subject, and the excellent bibliography which is provided will be invaluable to him for further reading. J. M. EDMONDS

Mammalian Biology

Mammalogy. By Terry A. Vaughan. Pp. viii+463. (W. B. Saunders: Philadelphia, London and Toronto, 1972.) £6.20.

THE first fourteen chapters of this attempt at a single volume presentation of the biology of mammals comprise an adequate, and in places lively, account of mammalian form and species diversity. The orders are tackled systematically and each chapter is well illustrated. The remaining eight chapters deal with such generalia as ecology, zoogeography, behaviour, reproduction, metabolism and temperature regulation, water regulation, acoustical orientation and general conservation problems. These chapters are rather less than totally adequate, but perhaps this shortfall is the inevitable outcome of an attempt to cover too vast a field in too little space.

Mammalogy is written for the American market and aimed at college and university students. European students may experience difficulty in coping with the American vernacular, names for scientific names are not invariably given. The reader may be puzzled by the rather arbitrary choice of topics singled out for elaboration in the latter part of the book. For example, why is a whole chapter (which, incidentally, happens to be very good) devoted to acoustical orientation and specializations of the ear, but only one page to olfactory communication? Being on average nineteen pages long, each chapter allows only a glimpse at the field and there are, naturally, disappointing omissions. In the chapter on behaviour a discussion on the mechanism bringing about observed behaviour and a fuller treatment of the problems concerning innate and learned behaviour would have been welcome. The final chapter on mammals and man is very short and adds little to what every student knows —that the future survival of many mammal species is in the balance.

Expensively produced and well illustrated this book can hardly be recommended as a "must" for European students who tend not to study subjects like mammalogy *per se*, but their teachers may find it a convenient, though limited, reference book.

D. MICHAEL STODDART

CORRESPONDENCE

Nuclear Defence

Sir,—I supported wholeheartedly your editorial "France should not test" (*Nature*, **243**, 50 ; 1973), but I think that perhaps you were a little unfair in your remark that the French Government would be well advised to admit that its *force de frappe* is no more than an illusion.

I would, of course, agree that for its advertised purpose as a contribution to the defence of Europe against possible Soviet aggression, its short range and small size make it utterly insignificant if the United States is involved, and, of course, like our nuclear force, more likely to induce preventive attack than to act as a defence if the Russians could be sure that the Americans would not be involved.

I am surprised, however, that you have taken its advertised object at its face value, rather than as the window dressing which it seems to me to be. It is surely easy to believe that the real concern of the French general staff is still, as it has always been, with Germany rather than with Russia. This is not even any reflexion on the general well-meaningness of the present West German Government. Governments have changed in this respect in the past, and anyway, general staffs concern themselves with what is possible rather than with what governmental intentions are likely to be. Without nuclear weapons France could offer little resistance to a revanchist Germany and might well doubt the willingness of the United States to destroy such a Germany on behalf of France, and in doing so, destroy their main bulwark in Europe against the USSR.

Ludicrously inadequate as it would be in face of a Russian attack, the *force de frappe* is in both range and power entirely adequate as a deterrent to a Germany which does not possess such nuclear weapons. One could well blame the French general staff for taking the same narrow and limited view of their country's interests as is taken by all other national general staffs, but I think that it is unfair to blame them for suffering from illusions.

Yours faithfully,

J. H. Fremlin

Department of Physics,
Chancellor's Court,
The University of Birmingham,
PO Box 363, Birmingham B15 2TT

Reaching for the Stars

Sir,—I feel obliged to respond to the two articles "Eye on the Future" and "The Search for Signals from Extraterrestrial Civilizations" (*Nature*, **241**, 363 and 379 ; 1973).

The first of these, an excellent brief review of the Project Cyclops report[*], ends by questioning the cost and value of the search for extraterrestrial intelligence: "The scale of expenditure suggested for Cyclops, however, runs at four times the total annual expenditure of the British Science Research Council. Perhaps the question is whether it is more efficient or more satisfying to spend $600 million a year asking the stars for, say, a cure for cancer or to spend the same sum on cancer research on Earth." These peculiar comparisons ignore the difference in the potential support base and imply that a cure for cancer would be a major, if not the only, benefit of interstellar contact. Ideally Cyclops would be an international effort; but even excluding this possibility, the $600 million/year (during the construction phase only) is only 8 per cent of the 1966 US space budget and 20 per cent of that for 1973.

Conceivably a cancer cure could be discovered out of interstellar contact (assuming our biochemistry is not too different from theirs), but is only one of a host of potential benefits, many of which may be far more important. Any civilization we contact will be at least as advanced as we and probably much more so. In all probability they will have faced and solved many of the problems that beset the world today such as pollution, depletion of natural resources, population control and the assurance of continued genetic evolution in a compassionate society, to name only a few.

Present theories of galactic evolution and of the origin and evolution of life support the belief that intelligent civilizations have existed in our Galaxy for a few billion years. Interstellar communication may therefore also have existed for aeons, and our first contact could put us in touch, not with another isolated civilization, but with this galactic community. We would then have access to a galactic heritage of knowledge that might include, in addition to a five billion year pictorial record of the universe, the Galaxy, and its life forms, some insight into the common cause of intelligent life and its future role. Interstellar contact may well mean childhood's end for the human race. With what other outcome can this be compared?

In the second article James C. G. Walker analyses the consequences of using a type of search strategy that was known to be inadequate before the Cyclops study began. Walker rejects omnidirectional beacons on the grounds that "the power requirement of an isotropic call signal detectable at 100 light years is approximately equal to the world's present total power consumption". He then assumes beamed beacons that illuminate any particular receiver for a short fraction of the time and, not surprisingly, arrives at very pessimistic figures for probable search time. The Cyclops study assumes that the total cost of achieving contact should be minimized by balancing the costs of transmitting and receiving. This leads to receiving antennas several km in diameter, which can be realized as filled phased arrays. Omnidirectional beacons are then quite feasible and with these the search times are greatly reduced as indicated in Table 1.

Table 1 Search Times for Galaxies, using Walker and Cyclops Strategies

Average separation of communicative civilizations	Duration of search Walker strategy	Cyclops strategy
24 light yr	14 centuries	1 d[†]
240 light yr	14 million yr	1 yr
2,400 light yr	140 aeons[‡]	370 yr

[†] Needed to get complete sky coverage; otherwise 8 h.

[‡] Should be reduced, galactic disk is only about 1,500 light yr thick in solar neighbourhood.

A Cyclops receiving system with an effective clear antenna aperture of 5 km would detect in 1,000 s a coherent signal having an incident flux of 5 photons s^{-1} mile^{-2}[§]. It would detect a 16 megawatt omnidirectional beacon at 100 light years or a 1,600 megawatt one at 1,000 light years range. Even this latter beacon radiates less than one four-thousandth of the world's present power consumption from fossil fuels, and could be powered with a single modern nuclear plant. Man will qualify for participation in the galactic community when he is willing to invest his share in a balanced system, rather than expecting the other civilizations to do the whole job.

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[*] Copies available from John Billingham, NASA/Ames Research Center Code LT, Moffett Field, California 94305.

[§] Since these are 1.5 GHz photons an optical system of equal power sensitivity would have to be able to detect one photon s^{-1}/hundred thousand square miles.

Yours faithfully,

B. M. OLIVER

Plus ça Change

SIR,—Important decisions about the future development of atomic power must frequently be made by people who do not necessarily have an intimate knowledge of the technical aspects of reactors. These people are, nonetheless, interested in what a reactor plant will do, how much it will cost, how long it will take to build and how long and how well it will operate. When they attempt to learn these things, they become aware of confusion existing in the reactor business. There appears to be unresolved conflict on almost every issue that arises.

I believe that this confusion stems from a failure to distinguish between the academic and the practical. These apparent conflicts can usually be explained only when the various aspects of the issue are resolved into their academic and practical components. To aid in this resolution, it is possible to define in a general way those characteristics which distinguish the one from the other.

An academic reactor or reactor plant almost always has the following basic characteristics: (1) It is simple. (2) It is small. (3) It is cheap. (4) It is light. (5) It can be built very quickly. (6) It is very flexible in purpose ("omnibus reactor"). (7) Very little development is required. It will use mostly "off-the-shelf" components. (8) The reactor is in the study phase. It is not being built now.

On the other hand, a practical reactor plant can be distinguished by the following characteristics: (1) It is being built now. (2) It is behind schedule. (3) It is requiring an immense amount of development on apparently trivial items. Corrosion, in particular, is a problem. (4) It is very expensive. (5) It takes a long time to build because of the engineering development problems. (6) It is large. (7) It is heavy. (8) It is complicated.

The tools of the academic-reactor designer are a piece of paper and a pencil with an eraser. If a mistake is made, it can always be erased and changed. If the practical-reactor designer errs, he wears the mistake around his neck; it cannot be erased. Everyone can see it.

The academic-reactor designer is a dilettante. He has not had to assume any real responsibility in connexion with his projects. He is free to luxuriate in elegant ideas, the practical shortcomings of which can be relegated to the category of "mere technical details". The practical-reactor designer must live with these same technical details. Although recalcitrant and awkward, they must be solved and cannot be put off until tomorrow. Their solutions require manpower, time and money.

Unfortunately for those who must make far-reaching decisions without the benefit of an intimate knowledge of reactor technology and unfortunately for the interested public, it is much easier to get the academic side of an issue than the practical side. For a large part those involved with the academic reactors have more inclination and time to present their ideas in reports and orally to those who will listen. Since they are innocently unaware of the real but hidden difficulties of their plans, they speak with great facility and confidence. Those involved with practical reactors, humbled by their experiences, speak less and worry more.

Yet it is incumbent on those in high places to make wise decisions, and it is reasonable and important that the public be correctly informed. It is consequently incumbent on all of us to state the facts as forthrightly as possible. Although it is probably impossible to have reactor ideas labelled as "practical" or "academic" by the authors, it is worth while for both the authors and the audience to bear in mind this distinction and to be guided thereby.

Yours faithfully,

H. G. RICKOVER

This previously unpublished comment on the practical problems of nuclear power was originally written in June 1953 but is thought still to be relevant today.—Editor, *Nature*.

Announcements

International Meetings

June 13, **Radioisotopes in Haematology** (General Secretary, The British Institute of Radiology, 32 Welbeck Street, London W1M 7PG).

June 14, **X-ray Image Intensifier Systems** (General Secretary, Institution of Electronic and Radio Engineers, 8 Bedford Square, London WC1B 3RG).

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NATURE Volume 243 JUNE 8 1973

The Janus in the Soviet Camp

THAT science and politics are inextricably mixed in the Soviet Union is well known, especially to those who have regularly followed the pages of *Nature* in recent years. But in the past twelve months there has been evidence of an increased thaw in relations between the Soviet Union and the western world and it is sad that yet once again an event has occurred which is bound to set back the processes by which amiable interchanges between the Soviet Union and the West have increased.

The incident which has now cropped up concerns the treatment accorded Dr Evgeny Levich, son of Professor Benjamin Levich, and if all the details which have emerged from the Soviet Union are substantiated, it represents a complete reversal to all that was bad in the Stalinist era. Evgeny, whose trials began in March 1972 when he applied for permission to emigrate to Israel, has recently been whisked away to Tiksi within the Arctic circle to start a period of military service. But the unsavoury aspect of the entire affair is the way in which this was done without any apparent regard for Evgeny's medical condition. A year ago (see *Nature*, **238**, 365 ; 1972) it was reported that he had a serious blood pressure condition but according to a recent telephone message from Professor Benjamin Levich his son's condition has now been diagnosed as being caused by ulcerous colitis and multiple diverticulosis of the bowels. As if this is not enough Evgeny is also reported to have a rectal tumour whose nature has yet to be described. This then was the condition of the young Levich when on May 16 he was apprehended on the street by what can only be described as a press gang which took him away leaving his wife helplessly behind.

But what can now be done? Professor Levich hopes that by means of a massive outcry from the scientific community outside the Soviet Union the authorities will be forced to return his son from Siberia to Moscow both to receive further medical treatment and to continue his work as an astrophysicist at the Chemical Physics Institute of the Academy of Sciences. But it must be asked whether this is a pious hope? Professor Levich himself feels that his position has been bettered within the Soviet Union following just such a campaign which was launched a year ago on his behalf when he was deprived of his university chair and of his position as head of the Institute of Electrochemistry in Moscow following his application—along with his sons—for permission to emigrate to Israel. It is true that recently Professor Levich's right to publish in the open literature has been restored but now there is news that he is faced with expulsion from the Soviet Academy of Sciences, on the grounds that he had engaged in activities harmful to the Soviet Union. These activities, apparently, were his refusal to protest against the efforts being made in Britain and elsewhere to publicize his case. These included a press conference called last summer by scientists who had met Professor Levich in Moscow and an appeal launched on his behalf by Professor D. B. Spalding of Imperial College. Professor Levich, however, has had neither his chair nor his position as head of the Institute restored since the campaign began.

In spite of this superficial lack of success, has the campaign in fact prevented Professor Levich's plight worsening? Professor Levich himself is convinced that much worse would have befallen him if it were not for the interest his case has raised outside the Soviet Union. From the position of an onlooker, in which the rest of the scientific community necessarily finds itself, it is difficult to argue with this logic. It now seems that a much better test of the sensitivity of the Soviet authorities to outside pressure will be found in the case of Evgeny Levich. Here, at least, the aim is straightforward, namely to have him certified medically unfit for military service. The campaign will have been a success if Evgeny is indeed returned to Moscow for treatment and to resume his career.

But only time will tell if an all out campaign of publicity is the best way to help Evgeny Levich. What is unfortunate though is that it is highly unlikely that the many scientists working in the Soviet Union who are regular readers of this journal will have the opportunity of seeing this comment on the plight of one of their colleagues. It is only six months since *Nature* published proof that severe censorship of this journal takes place before it is circulated within the Soviet Union (*Nature*, **240**, 372 ; 1972) and from other accounts it is an unknown occurrence for the journal to appear regularly week after week. If the Soviet authorities are really intent on making a success of the various formal and informal exchange arrangements which are now in existence between them and other countries they must show that they are willing to do away with some of the restrictions placed on the scientific community which have persisted since the days of Stalin.

100 Years Ago

Hail Storm

DURING the passage across us this afternoon of a thunderstorm moving at so great a distance above the earth that the thunder was very feeble and the lightning very faint, we had a great hail storm, which commenced with conical-shaped opaque stones of the size of peas, at 4^h 27^m (only lasting one minute), beginning again at 4^h 29^m with circular transparent stones having a small opaque nucleus (again only lasting one minute), followed at 4^h 33^m with flattened stones of the form of common acid drops, transparent, except a thin opaque envelope (which soon melted), and having *externally* in the centre a small rugged piece of ice.

E. J. LOWE

Highfield House Observ., Nottingham, June 3

From Nature, **8**, 121, *June* 12, 1873.

OLD WORLD

New Hope of Beating Car Exhaust Emissions

For the past six months the National Engineering Laboratory and Shell have been driving a 1.8 litre Morris Marina around Britain whose carefully measured exhaust emissions almost meet the federal standards originally set in the United States for introduction in 1976.

After two and a half years work the two organizations have produced a device labelled Vapipe (heat pipe vaporizer) that, when fitted to a standard car, can reduce emission of carbon monoxide and oxides of nitrogen to the 1976 levels of 3.4 and 0.4 grams per mile respectively, although it has no effect on hydrocarbon emissions.

The technique is neat and simple, and the Department of Trade and Industry —NEL's boss—is already eyeing the export market.

The solution proposed aims to prevent the problem of auto emissions rather than cure it, and it is based around a system that Shell demonstrated last year (see *Nature*, **236**, 194 ; 1973). When the air/fuel ratio of a car is increased from its usual level of 15:1 to about 22:1 the flame temperature in the cylinder is reduced, and as a result, so is the production of CO and NO_x. But for a car to run at such a high ratio a perfect mixture of air and petrol is needed, or the car will backfire.

The principle of reducing emissions by this method has been established for some time, but the difficulty has been to find a method compact enough, and cheap enough to manufacture in bulk, that does not require large amounts of engine redesign.

One of Shell's early attempts worked quite well, but two tons of laboratory equipment were involved. But now, by the simple use of a heat pipe, the problem has been partially solved.

A closed tube is run from the exhaust pipe of the car to its carburettor. The pipe contains a fluid which is boiled by the heat from the exhaust gases and which then condenses at the carburettor end of the tube before flowing back to be reboiled. The heat at the carburettor end vaporizes the petrol into the air stream which has just passed through the carburettor and is about to enter the engine. Because it is vaporized the resulting petrol and air mixture is uniform and high air/fuel ratios can be achieved.

The system is not without its problems. The increase in air/fuel ratio inevitably involves a loss of power, but this is balanced partially by more efficient combustion. Nonetheless, larger engines may be the order of the day. Against this has to be put a marked improvement in miles per gallon and a fall in octane requirements. One test car has even been run on lead-free petrol.

One distinct advantage of the system over other possible solutions is cost. No one is prepared to quote an actual price, but the system is said to be considerably cheaper than the figure of £120 to £250 that has been put forward in the United States as the expected cost of cleaner cars.

The vaporizer still leaves the problem of hydrocarbons to solve. Engine redesign or catalysts seem the obvious answer, and it is possible that the new NEL and Shell system could be fitted

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NUCLEAR POWER

Shell Buys In

The Royal Dutch–Shell group has bought a \$200 million stake in nuclear power. In a deal completed in New York this week Shell has gone into a 50–50 partnership with the Gulf Oil Corporation in Gulf Energy and Environmental Systems Company (Gulf E and ES), the California based nuclear power division of Gulf.

Shell's money has bought it into the development of the High Temperature Reactor that Gulf has developed since 1967 at a cost of about \$250 million. The company already has orders for six HTRs on its books, even though its first full scale prototype—a 330 megawatt reactor at Fort St Vrain has not yet gone critical. The orders—totalling 5,400 MW—should provide Gulf and Shell with the start of a commercial return for their money by the beginning of the 1980s. The nuclear fuel side of Gulf's business should also provide a return by the end of this decade.

The move by Shell reflects two things. First, its growing awareness that the future of oil is limited, even if there are enormous profits still to be made, and second, its faith in the future of nuclear power. Although Gulf has only been in the nuclear business for six years (it bought out General Dynamic's nuclear division in 1967), its work on HTRs has by-passed the light water reactors, and the bulk of Gulf's interest is concentrated in HTRs, ancillary equipment, and the development of fusion.

Although Shell's initial investment is \$200 million dollars, if the HTR sells as well as the two oil giants expect, Shell's investment could increase significantly. Gulf already holds option orders on two more 1,160 MW units, and Gulf E and ES has already concluded agreements in West Germany and France whereby their HTR designs will be marketed, built and fuelled in Europe by French and German companies.

British Nuclear Design and Construction, one of the two nuclear consortia in Britain that are to be merged into one company under Sir Arnold Weinstock's GEC, have had close links with Gulf for some time. Twelve BNDC engineers visited Gulf's stations last year, and exchanges of information on pressure vessels and gas technology—which BNDC knows a lot about with its experience of building AGRs—have taken place. Taylor-Woodrow, a member of the BNDC consortia, already has a technical agreement with Gulf on pressure vessels.

Shell's choice of partner is partly one of necessity and partly one of skill. Neither Westinghouse nor General Electric, the two United States nuclear power giants, would be likely to want a new partner whereas Gulf, itself an oil company coming to terms with the future and calling itself an "energy" company, welcomed the additional investment and the use of Shell's worldwide contacts. The other advantage of joining Gulf is that HTRs have not been challenged on environmental and safety grounds to the same extent as the light water reactors which have been Westinghouse and GE's moneymakers.

Shell decided against starting off its own nuclear business, a spokesman said this week, because "extensive studies showed that we could not expect at this stage to develop our own nuclear technology on a reasonable time scale and on a competitive basis".

Apart from the HTR, Shell's stake in Gulf also provides it with an interest in the development of the fast breeder reactor, in the use of HTR as a source of process heat for industry, in light water fuel fabrication, and in direct cycle nuclear plants.

The agreement does not, however, include uranium mining and milling or enrichment services.

to existing cars with a catalyst added on to clean up the hydrocarbons, while new cars could be designed to produce fewer hydrocarbons with the new vaporizer included as part of the design.

If Vapipe does prove to be a commercial proposition, and if a suitably cheap catalyst to cope with the hydrocarbon emissions can be developed, then there must be a real possibility that the United States standards for 1975—which are easier to meet than the 1976 standards but which were still put back a year by Mr William D. Ruckelshaus, administrator of the Environmental Protection Agency (see *Nature*, **242**, 491 ; 1973)—will be introduced, in spite of the fervent opposition coming from Detroit.

CONSERVATION

Sargassum Saga

THE first attempts to clear the Japanese seaweed *Sargassum muticum* from the shores of the Isle of Wight have been successful. But now that the adult plants have been cleared, a two or three year campaign will have to be run to clear the young plants that are already springing up round the coast.

The seaweed, which is not indigenous to Britain, was first discovered in February by members of Portsmouth Polytechnic (see *Nature*, **243**, 231 ; 1973). Experience in British Columbia where the seaweed arrived in the 1940s and spread rapidly down the coast, lead to fears that it might also grow uncontrollably in British waters, forcing out other marine life, interfering with shipping, and defacing beaches.

To combat this, a group of marine scientists decided to attempt to eradicate the plant before it took a firm hold, and two to three tons of the seaweed were removed during the low tides at the end of May and the beginning of June.

The problem now is how to finance the long campaign to remove the young plants that are springing up. Unfortunately the weed could not be removed before it entered its fertile period and spores from the *Sargassum* have escaped and young plants are already growing again at Bembridge, where the seaweed was originally discovered.

Sargassum grows thick and fast, at the rate of two centimetres a day. Parts of the Bembridge ledges that were clear last Thursday had young plants five centimetres long established by Sunday.

Dr Gareth Jones of Portsmouth Polytechnic, who has organized the eradication attempt, said this week that now all the adult plants have been removed from Bembridge, St Helens and Shanklin the need is to train about a dozen people to recognize the leafy young plants so that they can be cleared at fortnightly intervals when the tides are right in order to prevent the seaweed taking root again. At present of those who live locally and helped with the clearance only Dr Jones, and Dr Bill Farnham who discovered the *Sargassum*, are able to recognize the young plants which look rather different to the bushy adult weed which has been known to grow up to 10 metres long outside Britain.

The other problem is finance. With the help of teams from the University of Liverpool, North London Polytechnic and local sixth formers, the adult plants have been cleared at a cost of about £200. But Portsmouth Polytechnic can no longer afford to finance the eradication and more money is urgently needed. The most likely sources are the Nature Conservancy and the local councils on the Isle of Wight, but unless something like £500 a year can be found it will be difficult to ensure that the plant does not reestablish itself.

Dr Jones said this week that certain areas of the island—notably Totland Bay and Gunard Ledges—still need searching to ensure that adult plants have not grown up there.

Meanwhile the Natural Environment Research Council has given the Polytechnic a small grant with which to try and find out where the weed came from. Studies of the effect of *Sargassum* on other marine life—notably eel grass—will also be carried out.

The presence of *Sargassum* has highlighted a gap in government responsibility. Because it grows between high and low water marks, local government is not responsible for clearance, and central government can only be involved when the seaweed is proved to be a pest, by which time eradication would be impossible. Without the action of private individuals, no one would stop the weed establishing itself.

Short Notes

Windlesham succeeds Jellicoe

LORD WINDLESHAM has been appointed Leader of the House of Lords and Lord Privy Seal following Lord Jellicoe's resignation a fortnight ago. Lord Windlesham, a former director of Rediffusion Television and managing director of Grampian Television has been Minister of State at the Home Office since 1970. In succeeding to the job of Lord Privy Seal, Lord Windlesham will almost certainly take responsibility for the Civil Service Department, and may take charge of the changes that were introduced in last July's White Paper on Government Research and Development which Lord Jellicoe formulated and saw through Parliament, although the task of being the Government's spokesman on science does not necessarily fall to the Lord Privy Seal.

Stewart to chair ABRC

PROFESSOR F. H. Stewart, Chairman of the Natural Environment Research Council, is to succeed Sir Frederick Dainton as Chairman of the Advisory Board for the Research Councils. Sir Frederick was appointed Chairman of the University Grants Committee earlier this year.

Professor Stewart, who will take up his appointment in October, has been Chairman of NERC since 1971. He is also Regius Professor of Geology at the University of Edinburgh. In his capacity as Chairman of NERC, Professor Stewart already holds a seat on the advisory board, and he was a member of the ABRC's predecessor, the Council for Scientific Policy, from 1967.

Honours for Scientists

J. A. B. GRAY, Secretary of the Medical Research Council was one of five scientists knighted last week when the Birthday Honours List was announced. Dr Gray, who has been the MRC's secretary since 1968, was made a knight bachelor, as was Professor Raymond Firth, Professor of Anthropology at the University of London, Dr Percy Kent, formerly Chief Geologist and Exploration Manager to British Petroleum, Dr James Menter, Director of Research and Development at Tube Investments, Vice-President of the Royal Society and former President of the Institute of Physics, and Professor Eric Scowen, chairman of the Committee on Safety of Medicines. Earlier last week Sir Eric Ashby, Master of Clare College, Cambridge, since 1959, and former Chairman of the Royal Commission on Environmental Pollution, was made a life peer.

Professor Herbert Dingle : An Apology

IN *Nature* of January 12, 1973, we published a review by Professor John Ziman of Professor H. Dingle's book *Science at the Crossroads*. Though most of the review consisted of a discussion of ideas put forward in Professor Dingle's book, at one point in the review the book was described as "dishonest". We accept that there was no basis for attacking Professor Dingle's personal integrity and accordingly both *Nature* and Professor Ziman offer their sincere apologies to Professor Dingle.

NEW WORLD

Another Pesticide in Trouble

by our Washington Correspondent

THE series of battles that have been taking place in the United States over the safety of pesticides bears a strong resemblance to the interminable run of James Bond films: the same cast of characters appears in the same situations, and each successive production is as absorbing as its predecessor. Last year, the big attraction was the trial of DDT; this year, the trial of aldrin and dieldrin will soon begin its opening run in Washington. Unlike the James Bond films, however, there is more at stake than mere entertainment value, and the outcome is not always as predictable.

A trailer for the aldrin and dieldrin epic was given in Washington last week, when lawyers for the parties in dispute over the risks and benefits of the pesticides submitted their prehearing briefs to a hearing examiner. A public hearing will open soon—the date has not yet been decided—but the pretrial briefs outline the arguments which will be used by the Environmental Protection Agency and the Environmental Defense Fund, who want to get the pesticides off the market, and the Department of Agriculture and the Shell Chemical Company who want to continue using them. The public hearing will be the latest—perhaps the final—stage in a prolonged dispute over the pesticides.

The story so far is that in June last year, William D. Ruckelshaus, then Administrator of the Environmental Protection Agency, cancelled all registered uses of the pesticides except for mothproofing, termite control and some nursery uses. Shell, the sole manufacturer, objected to the cancellation notices and asked for a public hearing. The issues which will be debated are remarkably similar to those which came up in the prolonged dispute over DDT.

Aldrin is an organochlorine pesticide which is degraded fairly rapidly to the epoxide dieldrin, which is extremely long lived. It is used as a pesticide in both forms, and they will be considered together in the forthcoming public hearing. The chief use of the pesticide is to control pests in the soil to prevent damage to corn, and other uses in dispute are the treatment of seeds and the control of soil pests in the cultivation of citrus fruit in Florida. According to the brief from Shell, about 8 million acres of corn fields are treated with aldrin each year at a cost of about $1.50 per acre, and it is also used in about 30,000 acres of citrus. In all, some 10 million pounds of the pesticide is used each year, which is, less than the 20 million used in 1966.

In short, the problems with aldrin stem from the extreme persistence of its breakdown product, dieldrin, which leads to the familiar biomagnification in the food chain, alleged damage to wildlife and, what will undoubtedly be the crucial point in the hearings, its chronic toxicity to man. As with DDT, the most hotly debated topic is likely to be the possible cancer hazard.

Lawyers for Shell will argue at the hearings that the pesticide only poses a problem because of incorrect or careless usage. Their prehearing brief points out that when used to control soil insects in corn growing areas and in Florida, the pesticide is buried three inches below the surface, which should be sufficient to prevent it from being transported by agricultural runoff. They will also argue that there are no alternatives, and that there is no way of telling which fields are likely to be attacked by soil pests, hence farmers must use it before an outbreak as insurance.

But lawyers from the EPA will beg to differ. Their brief promises to reveal evidence of heavy concentrations in water, and in many aquatic species and in some birds. As further evidence of transportation, they will show that dieldrin is found in Antarctic penguins and whales. More important, an EPA study in 1971 turned up evidence of dieldrin residues in adipose body tissues of 99.75 per cent of the US population, and another study found residues in 83 per cent of the composites of meat, fish and poultry and 80 per cent of all milk samples consumed in the US.

Those figures will be the starting point for the EPA's arguments about the possible chronic toxicity of dieldrin to man. The evidence for possible carcinogenicity derives chiefly from long term feeding studies conducted on mice and one study (which the Shell brief curiously neglects to mention) conducted by the Food and Drug Administration on rats. Most of the key mice experiments were in fact conducted in Shell's own laboratories, but Shell and its opponents differ in the interpretation of the results, and the public hearing seems destined to develop into another debate about the applicability of animal studies to man. In many respects, the arguments are similar to those which continually circulate about the safety of food additives.

The EPA and EDF lawyers take the position that it is usually imposible to prove with absolute certainty that a chemical is carcinogenic to man, but if it is found to be carcinogenic to test animals then it must be regarded as a danger to man. As the EDF brief points out, "only a human catastrophe can prove conclusively that a chemical is carcinogenic to man". In the case of dieldrin, several mouse feeding studies have turned up evidence of liver tumours, while the FDA rat study suggested an increase in the incidence of tumours at various sites.

Shell's lawyers maintain, however, that the evidence of liver tumours in mice "says volumes about the mouse, (but) is not evidence of human tumourigenicity . . . the mouse liver tumours here are naturally occurring. Dieldrin merely increased their incidence". Shell backs up its case with data derived from a study of workers in an aldrin/dieldrin production plant, exposed to high concentrations of the pesticide. The study has turned up no evidence of liver tumours, or other signs of disturbance of liver function. But the EPA document points out that this study was carried out on "presumably healthy adults and the duration of their exposure is insufficient for an assessment of important chronic effects".

In many respects, the forthcoming hearings will boil down to a debate over the important philosophical distinction between socially accepted level of risk, and safe level for man. It should be remembered that in the case of DDT, the hearing examiner determined that use of that pesticide constituted a socially acceptable level of risk, and consequently recommended that DDT should continue to be used. Mr Ruckelshaus decided, however, that there was no safe level for man, and banned DDT. Whether the arguments over aldrin/dieldrin will be as finely balanced remains to be seen.

NIH

Unknown Quantity

by our Washington Correspondent

FOR the past few months, several scientists at the National Institutes of Health have been up in arms about the health policies of the Administration. Their disaffection first set in last December when Dr Robert Q. Marston was informed by the White House that he was being fired as the NIH Director, and it turned from bad to worse in January when the Administration's budget was unveiled to reveal savage cutbacks in a variety of biomedical research programmes. More recently, considerable alarm has been generated at NIH by a memorandum from the Office of Management and Budget attacking the peer review system by which grant proposals are evaluated for scientific merit (see *Nature*, **243**, 257 ; 1973).

It is thus a troubled agency that Dr Robert S. Stone inherited last week when he was officially confirmed as the new NIH Director. A pathologist who has been Dean of the School of Medicine of the University of New Mexico for the past five years, Dr Stone's appointment has not caused much of a stir at NIH. He is very much an unknown quantity and the general attitude there seems to be to wait and see. But some scientists polled last week said that they are at least happy that he has had some research experience (in tumour virology, etiology of lung diseases and development of a regional plan for meson radiotherapy of cancer), unlike a few of the candidates who were rumoured to have been in the running for the job.

One of the chief reasons for discontent at the firing of Dr Marston last year was that it is widely suspected that he was removed by the White House essentially because he voiced opposition to some Administration policies (no official reasons have ever been given) and Dr Stone's public statements and positions will be carefully watched to see whether he will toe the Administration line. At his first meeting with the press last week Dr Stone gave few things away.

He maintained several times that he has been given no instructions about policy from the White House or from the Department of Health, Education and Welfare, and said that he expects to voice his own opinions vigorously in the formulation of policy. As for a specific issue which has been troubling several people, namely that the National Cancer Institute and the National Heart and Lung Institute between them soaked up all the increase in the NIH budget while other institutes suffered cuts, Dr Stone merely said that the balance of funds between institutes and between programmes needs to be continually under review. He added, however, that "it is less important where the money is, as long as a substantial amount is going into fundamental research".

Turning to the question of training grants, Dr Stone did not say outright whether or not he agrees with the Administration's decision to phase them out entirely. The NIH is on record, however, as vigorously defending training grants last year in the preparation of the budget document, but it lost the battle to the Office of Management and Budget. Dr Stone acknowledged that training grants have been very important "in building up a cadre of scientists", and he added that if the question is reopened, he will "bring my viewpoint on what training grants have accomplished", which seems to come close to suggesting that he will fight for them to be reinstated.

Dr Robert S. Stone, New Director of the National Institutes of Health.

Finally, on the question of peer review, Dr Stone praised the accomplishments of the system and said that he is "clearly in support of peer review—I don't know what we can substitute for it". Specifically in regard to the OMB memorandum, he said "my bias is that scientists around the country have been reviewing each other's work with a high level of objectivity. I would be very careful about substituting anything for peer review".

Dr Stone brings to the National Institutes of Health considerable experience as a scientist-administrator. Born in 1922, he received his BA degree from Brooklyn College, New York, in 1942 and his MD from the State University of New York College of Medicine in 1950. He moved to UCLA School of Medicine in 1952 and in 1963 was appointed Chairman of the Department of Pathology at the University of New Mexico. He became Dean of the School of Medicine there in 1968, managed to attract good staff, and built it up during a difficult financial period.

Short Notes

Lunar Radio Astronomy

A SATELLITE designed to study low frequency radio signals from galactic and extra-galactic radio sources is set for launch at the end of this week. Called Radio Astronomy Explorer-B (RAE-B), it will be placed in lunar orbit and will study cosmic radio noise in the 0.02–13 MHz region. The satellite is a follow up to Explorer-38 which studied low frequency radio noise for four years after it was launched into Earth orbit in 1968. A lunar orbit was chosen for RAE-B because interference from radio noise from Earth saturated the receivers on Explorer-38 for between 25 and 40 per cent of its viewing time, and also solar activity after it was launched was greater than expected. The idea is that the Moon will shield the satellite from Earth radio noise, but the lunar orbit also has the advantage that lunar occulation can be used to pinpoint radio sources. The satellite, which cost $8.6 million, is due to be launched from the Kennedy Space Center on June 9.

TA in Commerce

THE Department of Commerce has set up a new Office of Technology Assessment and Forecast, chiefly to analyse and disseminate information contained in the files of the US Patent Office. A preliminary report published last week suggests that the premise underlying the new office's work is that changing trends in the patents filed at the Patent Office can be an accurate indicator of technological activity throughout the world. The office will thus seek to identify those areas of technology which have unusually rapid growth and also those areas in which a high proportion of patent activity originates outside the US. The idea will be to publish reports of patent activity in such fields. According to Dr Betsy Anker-Johnson, the new Assistant Secretary of Commerce for Science and Technology, other types of data, such as census reports and export and import figures, may be added to the system later. The office is being directed by Mr Alfred C. Marmor.

NSF to the Rescue

The National Science Foundation has granted a stay of execution to the Space Radiation Effects Laboratory in Newport News, Virginia. A 600 MeV proton accelerator operated for NASA by William and Mary College's Virginia Associated Research Center, SREL, was due to be closed down on June 30 because of lack of money. NSF is to put up $260,000 and William and Mary will put up another $145,000 to keep the machine running for another year, because the NSF considers it to be "an important intermediate energy facility". The accelerator was built in the mid 1960s at a cost of $15 million and it is used to simulate particle interactions in the Earth's outer atmosphere. The NSF grant only extends for a year, however, and in view of the failure of other accelerators to struggle on without federal grants, SREL's days are probably numbered.

NEWS AND VIEWS

Machine Nous

AFTER two months of "such limited studies as he was able to make", Sir James Lighthill (*Artificial Intelligence*, SRC, April 1973) has rejected the claim of artificial intelligence to special consideration by the Science Research Council on grounds which differ remarkably little from Lady Lovelace's objection to Babbage's analytical machine in 1842 (Turing, *Mind*, **59**, 433; 1950). Apparently it is as true today as it was more than a century ago that what you get out of a machine depends on what you put into it: as Lady Lovelace has it, "The Analytical Engine has no pretensions to *originate* anything. It can do *whatever we know how to order it* to perform"; or according to Lighthill, modern machines can successfully ". . . perform in highly specialized problem domains, when the programming takes very full account of human experience and human intelligence within the relevant domain . . .".

He goes on, however, to describe as the "coveted long-term goal of AI" the development of "a general-purpose program seeking to mimic the problem-solving aspects of human CNS activity". It is on the grounds of what he feels to be inadequate progress towards this goal that Lighthill concludes that further support for basic research on artificial intelligence as such—that is, as distinct from advanced automation or theoretical neurobiology—is unjustified. This immediately exposes him to the criticism that mimicking human intelligence is not the goal of artificial intelligence research, which is concerned with intelligence *per se*, and not necessarily as implemented in the human brain.

Be that as it may, it is entirely understandable that an outsider should come away with the impression that mimicry is the ultimate aim, when according to many insiders the crowning achievement in the field so far is Winograd's program for conversing with a machine in natural language (see *Nature*, **242**, 372; 1973; and Winograd, *Understanding Natural Language*, Edinburgh University Press, 1972). The excitement engendered by this development is acknowledged by Lighthill in a postscript considering separately the claim made for the program as vindicating artificial intelligence research.

The success of Winograd's program in dealing with the complexities inherent in the ordinary use of English depends partly on its having access to information on the universe of discourse: its behaviour is guided by the context within which it is working, as would be the case with human behaviour, and not simply by the input and its own intrinsic logic. In spite of this gift of human nous, Lighthill points out, it took a large computer a long time to make simple conversation about coloured blocks on a table top; and Lady Lovelace's objection still stands.

Thus Lighthill concludes that the results of work on artificial intelligence remain disappointing, and adds, more contentiously, that its goals are as remote as ever: a guess which is presumably based on Lighthill's general impression of the quality of work in the field. Professor N. S. Sutherland, in a cogent if somewhat waspish critique of Lighthill, raises the possibility that his impression is a function of the singers rather than the song. Sutherland bluntly states, "There is little first class work on basic AI in progress in Britain at the moment"; the leading workers in the field are mostly in the United States at Stanford or the Massachusetts Institute of Technology, and according to Professor D. M. Michie, who also submitted his comments, Lighthill did not actually speak to them.

Sutherland argues vehemently the importance of studying intelligence without the constraints of its mechanism in living brains (which, he points out, can generate some extremely unintelligent behaviour). In concrete terms, artificial intelligence research has produced some extremely sophisticated programming techniques which Sutherland believes have general application. Lighthill does not dispute the contribution of AI research to the understanding of natural language; on the same principle, Sutherland emphasizes the insight into the processes of thought and perception gained by the attempt to formalize them.

As far as Sutherland is concerned, the source of most that is good is the group at MIT headed by Marvin Minsky and Seymour Pappert, within which the ideas and techniques on which Winograd's program is based were developed. This view is reflected strongly in his suggestions for the deployment of research funds. These include importing young workers from the United States, the provision of computers which can accept US software, and sending young Britons for training to MIT. One role which Sutherland envisages for basic AI centres is in the training of young workers to supply the other related research areas of advanced automation and theoretical investigation of the central nervous system.

It is fair to say that progress in artificial intelligence has been unamazing; but in the twenty or so years that have elapsed since Turing considered the question of whether machines could think, and concluded with the suggestion that a machine might be built to play chess, or to understand English, machines have been developed that play chess, albeit only at the level of the talented amateur; and now they can also speak English, if only with the voice of the programmer. Progress in the next twenty years will doubtless depend on financial support for workers of Turing's calibre.

Probing in the Rain

THE increasing demand for telecommunications capacity in Earth-satellite and terrestrial networks requires the extension of present services to carrier frequencies above 10 GHz. Not only do new channels then become available but practical bandwidths are compatible with the needs of high-speed transmission of digital information.

An important problem in the development of the new services is the attenuation of the signals with absorption and scattering of energy which occurs due to precipitation in the transmission path. Rain, for instance, is highly variable: it may fall at a low rate as in drizzle or at a high rate in thunderstorms, be widespread over an area or of limited extent in showers, and its intensity and duration differ considerably from place to place and from one occasion to another. This variability makes it difficult to obtain good estimates of the reliabilities likely to be achieved by particular millimetre-wave communication systems. Transmissions must be maintained for a high proportion of the time, and the systems must remain operational through all but the extreme conditions of precipitation which may occur very rarely.

Although measurements of rainfall have been accumulated for many years now, they often refer only to the total amounts falling at different places during intervals which may be as long as a day and are rarely less than two minutes. They are not easily converted to indicate the near-instantaneous variations in all the rain affecting a radio transmission path. Investigations are therefore in progress in many countries to collect new information relevant to the transmission problem and to find ways of utilizing the long-term records.

One method of acquiring information is to use the Sun as a source of millimetre radio-waves and to measure the attenuation caused by rain in the path. Statistical distributions of the probability that the attenuation will exceed specific levels may be obtained at different frequencies, but measurements at one frequency or at separate frequencies at different times do not give the variations of rainfall intensity along the path uniquely. Extrapolation to other frequencies is doubtful and the sizes of the rain cells are not directly deducible.

In the article by Hogg on page 337 of this issue of *Nature*, simultaneous measurements of attenuation at 16 GHz and 30 GHz on Earth–space paths using a Sun-tracker in New Jersey provide statistical estimates of the intensity and extent of rain showers during the daytime periods of one year. The same antenna was used for reception at both frequencies so that the attenuations at each frequency at any time arose from the same rain shower. The absolute attenuations and the corresponding ratio of the attenuations at each frequency were known for any percentage of the total observing time. The ratio of the attenuation at 30 GHz to that at 16 GHz varied with the value of attenuation at 16 GHz. Assuming that the rate of rainfall was constant over part of the path and that the distribution of the raindrop sizes was the commonly-used one of Laws and Parsons, the attenuation ratio was calculated from theory for a range of rainfall rates and found to vary in a similar way to the variation of the ratio found experimentally. It was thus possible to relate the apparent rainfall rate occurring on the path to the attenuation at 16 GHz for any percentage of the recording time. As the attenuation coefficient is known theoretically, the path length through the rain at a specific apparent rate was deduced from the absolute value of attenuation.

In this way a statistical relationship was obtained between the apparent rain rate on the path and the extent of the rain for any probability level. For New Jersey, cell sizes ranged from about 11 km at rates of 5 mm h^{-1} to less than 2 km at a rate of 100 mm h^{-1}.

The method enables observed statistics of attenuation to be extrapolated to give statistics at any other frequency where the assumed distribution of raindrop sizes is acceptable. And the information on the dimensions of rain cells is valuable for estimating suitable distances between earth terminals in path-diversity systems where switching between paths is used to avoid regions of rain producing large attenuations. This double frequency technique will also be suitable for communications on terrestrial paths.

From a Correspondent

PULSARS

The First Century

JODRELL BANK announced last week the hundredth pulsar. Code-named PSR 1831–04 (the numbers being an abbreviated form of its location in the sky), it was discovered during a survey to examine the distribution of pulsars in the Galaxy, in particular to see whether the distribution is as expected if pulsars are indeed formed in supernova explosions. With thirty-eight pulsars attributed to them, the Jodrell Bank group are the most prolific discoverers of pulsars, and they are particularly proud that the instrument which they use, the Mark I dish, is the smallest telescope to have found one.

ANIMAL BEHAVIOUR

Stress in Farm Animals

from a Correspondent

ALTHOUGH there is widespread public concern about possible stress and suffering in farm animals that may be caused by some husbandry practices, formulation of satisfactory legislation is hampered by an important unanswered problem. Is it possible to give an objective definition of suffering in animals? A symposium organized jointly by the Society for Veterinary Ethology and the Royal Society for the Prevention of Cruelty to Animals at the Zoological Society of London on May 25 and 26 considered the whole problem of defining and diagnosing stress in farm animals.

Professor J. R. Napier (Birkbeck College, London), speaking on behalf of the RSPCA, opened the symposium by suggesting that although ethological studies must ultimately be scientific and objective, a little cautious anthropomorphism could with benefit be used as an interim measure, to give hypotheses as to what other animals feel.

No unified or simple definition of stress emerged from the meeting, but several speakers agreed that there may be two sorts of stress. There are short-term reactions to alarming or dangerous stimuli which are a normal part of an animal's behavioural repertoire and which are part of its adaptive mechanism for self-preservation. For example, fear may help an animal avoid being eaten by a predator. But apart from these normal reactions, there may also be continuing and severe manifestations of stress which are abnormal and harmful.

Some physiological measures of responses to unusual or damaging stimuli were discussed as indicators of either long-term or short-term stress—heart rate, electrical activity of the brain or overt manifestation of disease—but there was general acknowledgment of the difficulties of relying on these measures. Physiological and behavioural measures of stress do not always go together and there may be important differences in the manifestations of stress not just between mammals and birds but between different strains and even individuals. Indeed, it may transpire that no one measure of stress is sufficient and that a totality of symptoms should be taken into consideration.

It was evident from the meeting that knowledge of the behaviour of domestic animals is inadequate. Man is faced with the choice of regarding them simply as producers of eggs and meat or of acknowledging that they, like human beings, have been shaped by natural selection to behave and to feel in certain ways.

BIOLUMINESCENCE

Identified Glow

from our Molecular Biology Correspondent

"I CAN think of nothing eerier/Than flying around with an unidentified glow on a person's posterior." Thus Ogden Nash, on the subject of the firefly. Little, one supposes, did he guess that the glow arises from the oxidation of the benzothiazole derivative, luciferin, by molecular oxygen after it has been bound to the enzyme luciferase and then adenylylated by ATP and magnesium. This much is now established, and it is also known that the dehydrogenated derivative, which contains one more double bond, dehydroluciferin, will compete with luciferin, and undergo the same chemistry, but for the last step, the enzyme-bound adenylate not being reactive to oxygen. It undergoes in the course of binding some interesting and revealing changes in fluorescence, and to see more deeply into the nature of the interaction of luciferin with the enzyme, Bowie, Horak and De Luca (*Biochemistry*, **12**, 1845; 1973) have now synthesized a further analogue, which does not react with ATP. This is the alcohol, in which the carboxyl group of dehydroluciferin is replaced by a hydroxymethyl group, and is referred to as dehydroluciferol. Its spectroscopic properties are similar to those of its analogues, and it binds with the identical stoichiometry and a similar association constant to luciferase. In the bound state it is unaffected by ATP. Reciprocal plots show strong inhibition of the luciferase reaction.

In the accompanying article, Bowie *et al.* (*ibid.*, 1852) examine the nature of its interaction with the enzyme. In the first place, it must be noted that the fluorescence spectrum of luciferin and its analogues in aqueous solution shows a maximum in the region of 550 nm, which is characteristic of the phenolate form, in which the aromatic hydroxyl group is ionized. The excitation spectrum, however, is that of the neutral unionized molecule. This situation is not unique, for acids tend to become stronger on electronic excitation, and the proton can then dissociate during the lifetime of the excited state, leading to emission from the ionized species. When, however, the molecule is bound to the luciferase, a second smaller maximum, at about 425 nm, appears in the fluorescence spectrum, which arises from the unionized form. Thus the loss of the proton in the excited state is inhibited by the environment of the active site. There is at the same time a blue-shift in the phenolate emission, and both these phenomena are easily enough interpreted in terms of the location of the chromophore in a hydrophobic cavity. When now ATP with magnesium is added, a large increase in the relative fluorescent intensity at 425 nm ensues, indicative of a change in the character of the binding site, or of an increase in the fraction of ligand bound. In fact it turns out that both factors contribute, for titration in the presence of ATP and the corresponding binding plot show that the binding constant in these conditions is up by a factor of ten, but the resulting increase in the proportion of bound ligand is insufficient to account for the intensity change. The conclusion is that the quantum yield of the fluorescence of the bound form is increased by a factor of two or more.

Bowie *et al.* have also tried the effect of AMP, which is known to stimulate the chemiluminescence. It leads to a blue-shift in the phenolate emission, and the phenol peak is totally expunged. The polarity of the site can be matched by a solvent of low dielectric constant, chloroform, in which the phenolate emission of dehydroluciferol, which can be provoked in this medium by addition of a base to act as a proton acceptor, appears at the same wavelength. The AMP evidently also causes a change in the location of interacting groups in the binding site, in such a sense as to facilitate the proton transfer process. To complete the picture, the fluorescence decay kinetics have been observed. The initial formation of the unionized excited state is followed in the first few nanoseconds by the appearance of the excited phonolate. The proton transfer rate is diminished in the luciferase complex, and still more in the presence of ATP. These rates are reflected in the phenol excited state lifetime, which is increased on ATP binding, but marginally decreased by AMP. The authors suggest that the very high quantum yield of the bioluminescence may well involve the protection of the emitting species from quenching processes, and from hydrolytic destruction, by the hydrophobic milieu.

Bioluminescence of course occurs not only in fireflies but also in some bacteria, fungi and various creatures of the deep. In some of the luminescent coelenterates the mechanism is more or less similar to the firefly, with oxidation of luciferin on luciferase by molecular oxygen. This occurs, for example, in the class Anthozoa. The complicating feature of this system, however, has been that the chemiluminescent emission *in vitro* occurs at 490 nm whereas the intact animal lights up green, with a maximum at 509 nm, and some vibrational structure around 540 nm. An explanation has been contrived in terms of energy transfer to another chromophore, and indeed the search for such an acceptor led to the isolation of a green-fluorescing protein. Anderson and Cormier (*J. Biol. Chem.*, **248**, 2937; 1973) reasoned that if the excitation transfer is to proceed with high efficiency, the luciferase and the fluorescent protein should be packaged together at high concentration. The supernatant of homogenates was then discovered to produce the 490 nm luminescence, whereas the pelleted fraction emitted at 509 nm. This particulate matter could be purified by sucrose gradient centrifugation, and was found to contain luciferase, green-fluorescent

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Membrane Properties of Tumour Cells

IN *Nature New Biology* next Wednesday (June 13) Nicolson enlarges on the differences in agglutinating ability between normal and transformed cells. It has been known for some years that a number of plant lectins, which specifically bind certain saccharides, will agglutinate transformed but not normal cells.

This was attributed at the outset to the exposure of "cryptic" binding sites in the process of transformation. Since then evidence has accumulated that agglutinability of the cells is determined by the ease with which the glycoprotein receptors are able to diffuse in the plane of the membrane, and create clusters that promote strong interaction with another cell.

This cluster formation can be observed by labelling with ferritin-bound or fluorescent anti-lectin antibodies. It has also been reported that tumour cells will agglutinate with concanavalin A, a typical lectin, at room temperature, but not at low temperatures. It would be expected that this effect results from a dependence of the fluidity of the membrane on the temperature, which is what Nicolson has now demonstrated.

Concanavalin A-treated cells labelled with fluorescent anti-concanavalin antibodies at 0° C show uniform surface fluorescence. Normal cells first incubated at 22 or 37° C and then cooled to 0° C look similar. On the other hand transformed cells treated in this way show fluorescent patches. Labelling at 0° C after fixation with formaldehyde inhibits the temperature-induced formation of clusters. Thus the distinctive behaviour of tumour cells towards lectins is evidently a function of a greater fluidity in the membrane bilayer, and this effect is not expressed at low temperature.

protein and all the luciferin sulphokinase, an enzyme responsible for mobilizing the luciferin from its stored sulphate derivative. There is also a calcium-triggered "photoprotein", representing another chemically related luminescent system. The luciferin and green-fluorescing protein are not easily extracted from the pellet and may well be associated with a membrane. The particles in the electron microscope do indeed show evidence of a unit membrane envelope, and are about 0.2μ across. They emit under the action of oxygen and calcium ions, and the kinetics of emission are described. Anderson and Cormier have termed their particles lumisomes, and have found them also in the other coelenterates that they have examined.

Luminescent bacteria also possess a luciferase, but the substrate in this case is reduced FMN, and an aliphatic aldehyde is also implicated. As in a large part of the bioluminescence field, the outlines of the chemistry have been defined by Hastings and his associates. Some of the properties of the interactions of FMN with the enzyme have now been studied by Meighen and MacKenzie (*Biochemistry*, **12**, 1482; 1973). They have shown that a negative charge on the flavine is essential, and that its distance from the ring is critical for the interaction. The binding site for this charge can be satisfied by a phosphate ion, which competes against reduced FMN. With a neutral flavine the phosphate, or other suitable anion, strongly stimulates the luminescence. The deduction then is that the binding of the anion at a specific site on the enzyme stabilizes a functional intermediate, the chemical nature of which has been inferred.

Hunting the Helix. Following my remarks last month about the relation between conformational characteristics and sequences in globular proteins, a reader has commented that the observations of Crawford *et al.* (*Proc. US Nat. Acad. Sci.*, **70**, 538; 1973) on hairpin turns were largely prefigured by Kuntz (*J. Amer. Chem. Soc.*, **94**, 4009; 1972), who also found that they occurred almost exclusively on the surface. This greatly increases the stringency of the turn as a structural constraint, and thus its predictive value.

Correction

IN the editorial "Knack or Knowledge?" (*Nature*, **242**, 494; 1973), the reference to Peart and Newman in the first paragraph on page 495 should be to the Institute of Physics Conference Series No. 16. The title of this book is *Radiation Damage and Defects in Semiconductors*.

ECOPHYSIOLOGY

Arctic Adaptations

from our Plant Ecology Correspondent

ONE of the aims of the plant ecologist is to be able to explain the geographical distribution of plant species in terms of their adaptation to climatic conditions. Particular emphasis has been placed recently upon the temperature requirements of enzymatic systems within the plant, especially those enzymes associated with carbon dioxide fixation. Photosynthetic rates are known to be temperature sensitive and it has been demonstrated by Wareing *et al.* (*Nature*, **220**, 453; 1968) that the activity of ribulose-1,5-diphosphate (RuDP) carboxylase is an important controlling factor of this activity in light saturated conditions. It is natural that this enzyme has received considerable attention from ecophysiologists.

Treharne and Eagles (*Photosynthetica*, **4**, 107; 1970) found a higher rate of activity at low temperature for this enzyme in Norwegian populations of the grass *Dactylis glomerata* than in those from Portugal. Similar results have been obtained with conifers along altitudinal gradients (see *Nature New Biology*, **239**, 65; 1972). Recent studies on several plant species from cold habitats, however, suggest that the response of photosynthetic rates to temperature is not always mirrored by the response of RuDP carboxylase activity.

Chabot, Chabot and Billings (*Photosynthetica*, **6**, 364; 1972) have found that populations of the Arctic-alpine species *Oxyria digyna* from Arctic environments (76° N) and from high altitudes in lower latitudes (37° N) all have RuDP carboxylase with temperature optima in excess of 45° C. The observed decrease in photosynthetic rate in this species at temperatures above 25° C cannot therefore be explained in terms of a falling activity on the part of the carboxylating enzyme.

Tieszen and Sigurdson (*Arctic Alpine Res.*, **5**, 59; 1973) have now come to similar conclusions when working with three Arctic grasses, *Alopecurus alpinus*, *Arctagrostis latifolia* and *Dupontia fischeri*, collected near Barrow, Alaska; in all of these species RuDP carboxylase activity was found to rise with temperature, reaching an optimum at 50° C. As in *Oxyria*, the photosynthetic rates of these grasses decline above a temperature of 25° C or less, and indeed the optimum temperature for their RuDP carboxylase activity is above their temperature compensation points.

The adaptation of photosynthetic mechanisms to environmental temperature conditions is evidently far more complex than can be explained by reference to the response of a single enzyme. Once again one must turn to other

Transformed Cells Express More of their DNA

IT has long been suspected that transformed cells, being extremely active metabolically compared with normal cells, would have a greater proportion of their genome open for transcription. Early estimates of the amount of DNA transcribed were obtained using hybridization conditions which provide information on transcription of the repetitive fraction of the genome only. In *Nature New Biology* next Wednesday (June 13), Grady and Campbell report some careful hybridization studies which give information about non-repetitive DNA transcription in normal and transformed mouse cells grown in tissue culture.

Two normal cell lines, AL/N and Balb 3T3, were compared with their virus transformed derivatives PY AL/N and SVT2. Nonrepetitive DNA was isolated under the standard conditions of 60° C, 0.12 M phosphate buffer using only the fraction of DNA which renatured at a $C_0t > 220$ and was shown to be essentially free of repeated DNA. RNA–DNA saturation hybridization experiments were evaluated by the double reciprocal plot method of Bishop *et al.* and the saturation values obtained were thus shown to be independent of the RNA concentration used.

Approximately 20% of the nonrepetitive DNA in normal AL/N cells is expressed in confluent (cells grown to their maximum cell density) and subconfluent cultures. When a mixture of confluent and subconfluent AL/N RNA was used it was found that the unique DNA sequences expressed in these two growth stages differed by as much as 5%. Saturation hybridization experiments with PY AL/N RNA showed that in virus transformed cells 30% of the unique DNA sequences is expressed in both confluent and subconfluent cultures. In this case, however, the regions of unique DNA transcribed in the two stages of growth were identical. In Balb 3T3 and SVT2 cell lines the amount of unique DNA sequences transcribed is again 20 and 30% respectively, in spite of large differences in chromosome number among all four cell lines.

Mixing experiments showed that the two normal cell lines differed by as much as 3% in the nonrepetitive DNA expressed and for the two transformed lines the difference was as much as 7%.

Grady and Campbell have thus presented firm evidence that virus transformed cells express more of their unique DNA and that the new sequences which are transcribed may vary from one transformed line to another.

factors, such as respiratory mechanisms and leaf resistance to the diffusion of carbon dioxide, for an answer to these seeming anomalies.

VIROLOGY

Sudden Death in Infancy

from a Correspondent

THE very sudden and unexpected death of a previously healthy infant—the so-called cot death—occurs more frequently than deaths in childhood due to road accidents. Yet the true cause of these cot deaths is rarely established although several theories are being examined. It may be caused by an overwhelming virus infection, or an allergic reaction possibly to regurgitated milk entering the trachea, or it may simply be that very young infants do not adapt sufficiently quickly to mouth breathing when their nasal passages are blocked.

A group of workers from Newcastle and Gateshead have studied fifty-one cases of sudden and unexpected deaths in infants (Ferris *et al.*, *Brit. Med. J.*, **2**, 439; 1973). They were looking for a link between virus infections of the respiratory tract and sudden death in infants under a year old, and therefore excluded all infants known to have been unwell before their deaths, those with any obvious defects such as congenital heart disease and those who at post-mortem were found to have any disease which could account for their death.

Their investigation of the fifty-one remaining unexplained deaths involved histological examination of lung tissue and attempts at virus isolation and identification. The viruses looked for were those known to produce respiratory illnesses in children—respiratory syncytial virus, influenza A, and para-influenza types 1 and 3.

Each slide of lung tissue was examined by two pathologists independently and complete agreement reached on their classification. The samples fell into three groups: the largest group of thirty-three cases showed changes of a type which have been widely accepted as characteristic of cot deaths, the main feature being a large volume of fluid in the alveoli of the lungs. These lung changes are similar to those found in children whose cause of death was thought to have been an acute allergic reaction. But in the Newcastle cases there seem to have been no obvious factors which could have provoked such reactions. No viruses were isolated from this group.

The second largest group contained sixteen infants whose lungs showed a breakdown in the lining of the smallest bronchial tubes and infiltration of lymphocytes. Viruses were isolated from thirteen of these cases, chiefly the respiratory syncytial virus.

Group three showed the appearances of bronchopneumonia, a bacterial infection. There were three children in this group, from two of which respiratory viruses were isolated.

So the chief point is that in a third of these cases of cot death viruses were isolated and seem to have been the cause of death because the viruses were not of the type expected in the lungs of healthy children. But clearly this is not the whole story, for it looks as if some kind of unexplained allergic reaction is involved in a proportion of cot deaths. Physicians are, however, no nearer a way of anticipating and preventing these tragic sudden deaths.

MARS

Mariner Results

from a Correspondent

THE Royal Society discussion meeting arranged by the British National Committee on Space Research on May 15 and 16 showed how drastically Mariner 9 has changed our view of Mars. Mariners 6 and 7 saw a cratered planet like the Moon except for some evidence of erosion, but Mars is now seen to be much more varied and to have many features in common with Earth.

A preliminary geological map of the whole planet, prepared from Mariner 9 photographs, is now available. Only half of the surface, mostly in the southern hemisphere, is substantially cratered. The surface in the south is more than 3 km higher than in the north and it is deduced that in the southern half of Mars the crust is ancient, low density, "continental" material. Thus about half of the surface of Mars is "continent", making it intermediate between Earth and Moon. It may be that the fraction of continental material might be a measure of the crustal stability of a planet.

It follows that martian material is not of uniform composition and so it seems that, like Earth and Moon, differentiation occurred early in the planet's history. In support of this view, spectral analysis shows the atmospheric dust to be 60% silica, and some very ancient volcanoes can be seen.

Volcanic activity that is geologically more recent covers a large area, and several volcanoes are similar to terrestrial shield volcanoes. The largest, Nix Olympica, is 700 km across and more than 24 km high. Three smaller shield volcanoes are aligned along the edge of the "continent". In the same region a vast canyon up to 6 km deep, 200 km wide and about 3,000 km long dissects the highest part of Mars. These observations suggest that some recent crustal movement of the type seen on Earth has occurred.

The entire surface has been modified by erosion and the most effective agent is wind. The martian atmosphere, almost wholly carbon dioxide, circulates due to differential solar heating, but it is only 0.5% as dense as Earth's atmosphere and so allows sharper temperature gradients to develop, resulting in generally stronger winds. Occasionally the winds become so strong that the lower atmosphere is filled with dust, although

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Archival Records Hint at Nature of OH471

AS expected, the discovery that the QSO OH471 has a redshift of 3.4 has caused a flurry of activity among observational astronomers. In *Nature Physical Science* next Monday (June 11), Kraus, Gearhart and Ehman present flux density data for the object going back to 1963, while in this issue of *Nature* (page 336) Wampler and colleagues report that they have found a redshift of 3.53 for the "blue stellar object" OQ172.

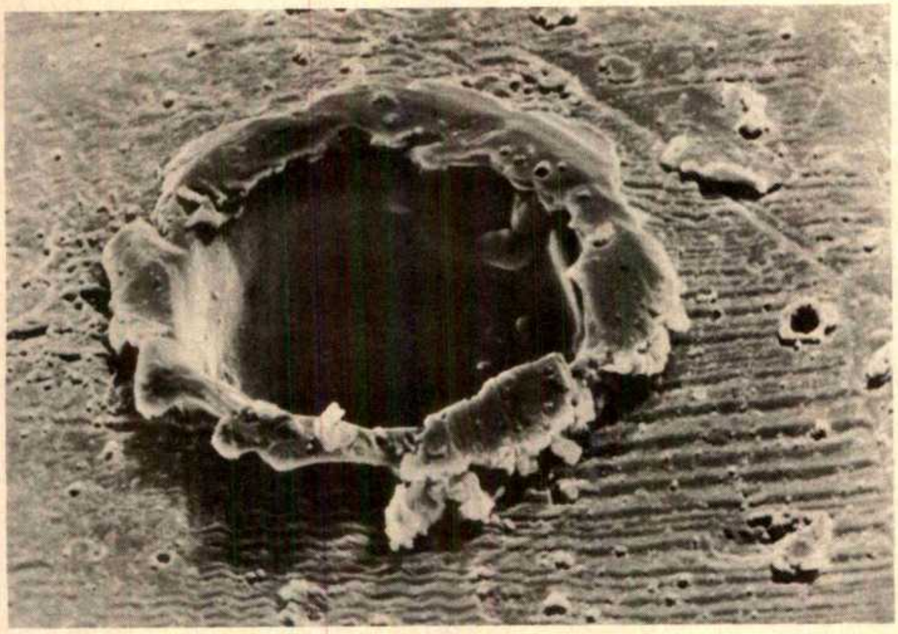
Clearly, the discovery of one high redshift object has indeed paved the way for other similar discoveries. But what of the observations of OH471 going back over ten years? It is certainly an almost unique event in radio astronomy that records of this kind have immediately been available for an object of newly discovered significance, although in the case of optical astronomy objects such as 3C273 can be examined on plates dating back to the latter part of the nineteenth century. The relevant radio data have been preserved, together with other data from many objects, in the Ohio State University Radio Observatory archives.

It is only a pity that such foresight has not been rewarded with more dramatic discoveries than that OH471 is not variable. The flux density has been measured at 1,415 MHz, 612 MHz and 2,650 MHz. With a density of about 2 f.u. at the lower frequencies and 1 f.u. at 2,650 MHz and above, the spectrum is of the centimetre excess (CE) type, and might have been expected to show variability.

The archival records dash these hopes; the range of the (averaged) flux densities is only from 3.2±1.2 f.u. to 2.09±0.6 f.u., which can be interpreted as indicating either no significant change or a slight decrease over the period 1963–70. Although this is slightly disappointing, the availability of these records will ensure that any variation of the source will become apparent much more quickly than it would if monitoring was only now beginning.

SELENOLOGY

Microcrater in Lunar Meteorite



This lunar crater is only three hundredths of a millimetre across. It was found in a 2 mm by 1 mm fragment of nickel-iron brought back from the Moon by the Apollo 16 astronauts. This specimen is part of a sample sent to the Max-Planck Institute for Nuclear Physics at Heidelberg, and the photograph was taken with a scanning electron microscope at the Siemens laboratory in Karlsruhe. The specimen is itself a fragment of meteorite and has in turn been struck by a micrometeorite which must have been travelling at more than 10 km s^{-1}. As well as the main crater the impact has produced a series of parallel structures in the surface of the sample, together with a smaller crater to the right of the main crater.

it is not known exactly how this happens. The dust storm in progress when Mariner 9 reached Mars became the longest and most severe ever seen. When the dust eventually settled into a layer possibly only a few millimetres thick the effects of aeolian action were seen everywhere. Slides were shown of features formed by wind in terrestrial deserts.

Craters interrupt the smooth flow of wind, causing the formation of dunes inside craters and long streaks downwind of them, and an analysis of streak directions has allowed the atmospheric circulations to be plotted, although only the abnormal conditions of a planet-wide dust storm may be represented. The importance of aeolian effects on Mars has stimulated modelling of erosion features in wind tunnels in the hope that spacecraft photographs may be more fully interpreted. Films of the erosion of craters in a wind tunnel were shown and obvious similarities with martian craters were seen.

Wind cannot account for all the erosion, however. In particular, channels are seen, some of them hundreds of kilometres long, which are morphologically similar to intermittent streams in terrestrial deserts. The meeting participants accepted that this implied the existence of huge quantities of flowing water at some time although water on Mars would quickly evaporate. A clue to the origin of these channels is the observation of areas of "cracked ground", characteristic of permafrost regions, near the heads of some of them. It seems possible that rapid melting of permafrost could have given rise to transient rivers but the origin of the permafrost is unknown.

Extreme dryness is the most serious impediment to the development of life on Mars. Consequently the two landing craft of the 1976 Viking mission, whose main object is the search for life, will be put down where water is most likely to have been present. One will land at a confluence of dry river channels and the other in the region with most water vapour at the time.

The polar regions of Mars also received attention. At each pole there is a region of "laminated ground" which is interpreted as a 6 km thick covering of layered deposits which are at present being eroded. The material removed is being redeposited in the zones north and south of 30° latitude which are covered by a smooth mantle and appear to be areas of deposition. In spite of the thick layer of deposits the poles are lower than their surroundings and this means that isostatic adjustment—the sinking of the crust under the extra load—has occurred.

This and the volcanic evidence indicate that the interior is warm and the crust relatively thin so that the idea of incipient crustal movement is plausible. Evidence against this is that Mars is not in hydrostatic equilibrium—this long suspected fact has been confirmed by Mariner 9. This could imply great internal strength but it may be possible to account for it if the interior of Mars, like the Earth's mantle, is undergoing thermal convection.

Link between Coronal and Interplanetary Shocks

CORONAL shock waves generated by solar flares are directly linked to interplanetary shock waves in the solar wind, according to evidence gathered by Pintér and reported in next Monday's *Nature Physical Science* (June 11). This follows the now well established link between type II solar radio bursts and flare-generated coronal shock waves as another step towards a more complete understanding of the Sun's activity as an overall phenomenon rather than a series of unrelated events.

Coronal shock waves are emitted by flares at the time of the triggering instability which can be observed in hydrogen alpha light; at the same time, the type II burst is initiated. Radio data have shown that the resulting shock moves out to at least 7 $R_\odot$, at a velocity of 500 to 4,000 km s^{-1}. At that distance from the Sun, the radio technique used becomes inadequate to monitor further expansion of the wave front. But satellite observations, notably from OSO-7, have now revealed the existence of plasma clouds, moving at velocities of around 1,000 km s^{-1}, at distances beyond 10 $R_\odot$ from the Sun. It now seems that such clouds represent coronal shocks which have escaped from the corona.

Pintér has shown the close relation between flare-generated coronal and interplanetary shock waves by comparing their velocities. Using two detectors (either two artificial space probes or one probe and Earth based instruments) at different distances from the Sun the velocities of the interplanetary shocks can be measured directly. All of those chosen for study were associated with type II bursts, and all were decelerating as they moved outwards. Extrapolating backwards, Pintér deduces the initial velocities of the shock waves, and finds that at 1 $R_\odot$ these are almost identical to the velocities of the coronal shock waves associated with the equivalent type II bursts, measured from the drift of the burst.

For average values of Pintér's data—initial velocity 2,000 km s^{-1}, velocity at 1 a.u. 530 km s^{-1} and particle density 10 cm^{-3}—the energy supplied by the flare to the shock is 6×10^{30} erg.

ASTRONOMY

Goddard Symposia

from Correspondents

THE Goddard Space Flight Center in Maryland was host recently to two international conferences: an X-ray astronomy symposium on April 27; and the first symposium and workshop devoted exclusively to gamma-ray astrophysics on April 27 to May 2.

X-ray Astronomy

Goddard seemed an appropriate place for the X-ray astronomy symposium because it was the project management centre for the first Small Astronomy Satellite (Uhuru) which has provided such a wealth of data on X-ray astronomy and has stimulated not only ideas for other X-ray experiments, but also interest in related measurements in optical and radio astronomy.

The first speaker at the morning session was Dr R. Giacconi (American Science and Engineering), the principal investigator of SAS-1, who began by announcing the publication of the third *Uhuru X-ray Source Catalog*. In addition to this catalogue, now containing a total of 161 X-ray sources, the position uncertainties for many of the sources previously listed in the second Uhuru X-ray catalogue have been significantly reduced. He described the large class of objects observed, including the binary systems. It now seems that the X-ray star of such binary pairs is sometimes quite massive and releases very large amounts of energy, and this has led to speculation that these particular objects are black holes.

Dr E. Kellogg (American Science and Engineering) described information on X-ray objects not in the galactic plane. He noted that the study of large clusters with redshifts of about 3 which are detectable in X-rays could give very deep insight into the evolution of galaxies.

Dr L. Peterson (University of California) noted that the energy spectra of X-rays associated with binary objects are often rather flat to 30 or 40 keV, at which energy the spectrum is observed to steepen sharply. No conclusion was reached regarding the most probable cause of this change in spectral shape.

Dr G. Garmire (CalTech), who concentrated on the soft X-rays, pointed out that nearly all classes of X-ray emitting objects discovered at higher energies by Uhuru are observed in the soft X-ray region. In general, soft X-ray observations can assist in determining distances to X-ray objects attributed to interstellar absorption. In addition, said Dr Garmire, future spectral studies of binary objects should lead to a detailed knowledge of the gas flow in these systems.

The afternoon session was devoted to the correlation of X-ray data with radio and optical observations and their relation to theoretical models for the sources. Dr R. Hjellming (National Radio Astronomy Observatory) spoke on radio emission from X-ray stars, Dr W. Liller (Harvard University) discussed the optical characteristics of X-ray sources and Dr K. Davidson (Princeton University) considered some theoretical aspects of X-ray source models, particularly for binaries. Although Sco X-1 is the brightest X-ray star in the sky, observed in the optical and radio bands as well, and the first to be discovered, Dr Davidson concluded that it cannot be explained in terms of a simple binary model as used for other X-ray stars. Hercules X-1, the source which is most nearly understood, is clearly a member of a binary system. The optical companion for this X-ray source has been well studied and its variations traced back about eight years by examining old Harvard plates. Other point X-ray sources exhibit less well behaved variability but have significant radio counterparts. Variations in the radio and X-ray bands have been correlated for the black-hole candidate Cygnus X-1. On the other hand, it was pointed out that the most pronounced radio variability observed, that for Cygnus X-3, did not correspond to any comparable variations in the X-ray band.

Gamma-ray Astrophysics

Important new results on the diffuse gamma-ray background as obtained by Apollo were presented by Drs L. Peterson (University of California, San Diego) and J. Trombka (NASA) and results obtained by SAS-II were presented by Dr D. Kniffen (NASA), who also reported observations of the galactic plane. The results from SAS-II confirm some important qualitative results first obtained by OSO-III that the Galaxy is an intense source of gamma radiation above 100 MeV and it stands out above the extragalactic background in this energy range. The spectrum is harder above 100 MeV than the gamma radiation seen at high galactic latitudes which is presumably extragalactic. The SAS-II results also indicate that the extragalactic (high galactic latitude) background spectrum is quite steep above 40 MeV.

Results from balloon observations by groups at the Max-Planck Institute and the US Naval Research Laboratory, reported by Dr G. Share (NRL), are consistent with the Apollo and SAS-II results which present a continuous observational spectrum from 300 keV up to 135 MeV. These data suggest a bulge in the gamma-ray spectrum above 1 MeV, in spite of background corrections which are of most importance below 4 MeV. This bulge has been interpreted as a new component of gamma radiation at energies above 1 MeV. This argument is even more important if the X-rays below 1 MeV are thermally produced and are falling off exponentially in energy above 100 keV as was suggested by Drs D. Schwartz (American Science and Engineering) and R. Cowsik (University of California, Berkeley). Problems with the thermal interpretation were discussed by Dr W. Kraushaar (University of Wisconsin).

The interpretation of the 1 MeV to 100 MeV bulge in the gamma-ray spectra was discussed by Dr F. Stecker (NASA), who concluded that the excess is most likely caused by matter/antimatter annihilation. The Apollo and SAS-II observational data present an excellent fit to the predicted annihilation spectrum up to 135 MeV. The matter/antimatter-symmetric cosmology was discussed by Drs R. Omnes (Laboratory of Theoretical and High Energy Physics, Orsay) and E. Schatzman and J. Puget (Paris Observatory, Meudon).

The exciting aspects of the matter/antimatter cosmology reported on by Drs Omnes, Schatzman, Puget and Stecker indicate that, in addition to implying baryon symmetry on a universal scale, it can explain such diverse phenomena as: the cosmic gamma-ray background spectrum; the ratio of photons to nucleons in the Universe of $\sim 10^9$; annihilation as the energy source for generation of large-scale turbulence leading to galaxy formation; and the consequent observed sizes, mean densities and rotational velocities of galaxies.

The galactic gamma-ray flux in the 100 MeV range seen by SAS-II and OSO-III indicates an increase in the direction of the galactic centre. The most likely implication is that there is a cosmic-ray gradient toward the galactic centre, as was pointed out in remarks by Professor A. Wolfendale (University of Durham) and in a communication by Dr V. Ginzburg (Lebedev Institute, Moscow).

Another interesting highlight of the meeting was the discussion of the 470 keV feature seen at the region of the galactic centre. Three interpretations of this feature were presented. Professor D. Clayton (Rice University), who discussed gamma-ray line emission, mentioned that it may be caused by lithium. Dr R. Ramaty (NASA) (who, with Dr D. Forrest (New Hampshire), discussed gamma rays from solar flares) suggested that this feature could be attributed to redshifted positron annihilation produced at the surface of neutron stars. (This has also been suggested by Guthrie and Tidemaru in *Nature Physical Science*, **241**, 77; 1973.) Dr M. Leventhal (Bell Laboratories) suggested that this feature may arise from a poorly resolved positronium annihilation spectrum.

Prospects for Cancer Research in Britain

This article sets out the comments of the Joint Cancer Research Campaign/ Medical Research Council / Imperial Cancer Research Fund Coordinating Committee for Cancer Research on Lord Zuckerman's recent report.

In a preface to Lord Zuckerman's report on cancer research in Britain (HMSO, October 1972), the Prime Minister invited the comments of the Coordinating Committee for Cancer Research as well as of other bodies. The committee represents the Medical Research Council, the Cancer Research Campaign and the Imperial Cancer Research Fund. Its comments on the Zuckerman report, now with the government, are as follows.

The committee agrees that "money alone will not buy the new galvanizing ideas that are needed". Such ideas spring from original minds, but these minds need to have had appropriate training and a suitable environment in which to develop. Moreover, new ideas are produced against a background of accumulating basic knowledge which, at the present time, is coming from a wide spectrum of activities spanning the whole field of biomedical research.

There is no evidence that, nationally, Britain is short of intellectual potential ; there are, however, inadequate opportunities to enable this potential to be brought to bear, and money is a limiting factor in providing these. If, therefore, it is desired to enlarge and accelerate the cancer research effort already going on in Britain, it is necessary to plan ahead for a long-term effort in terms of establishing more training and career posts for cancer research workers, providing new and better facilities for them, and ensuring, as the committee has already emphasized, that their work is carried on in close association with the main stream of other biomedical research.

Additional funds would enable a start to be made virtually at once in providing more training and career posts, but a limit would quickly be reached because of the lack of physical facilities, particularly accommodation. There is therefore a need to commit funds now to enable the planning of facilities to go ahead and, as these are provided, for example by the setting up of new oncological centres, so the number of posts should be further increased.

The committee makes two specific recommendations below about immediately foreseeable needs, that is, those on which it believes that money could actually and usefully be spent within a period of about two years from the time of commitment of new funds. Such a commitment would enable a start to be made in gearing up for an expanding programme. More funds would undoubtedly be needed beyond this initial period, but precisely how much is more difficult to forecast. This will depend on the degree of success in implementing the present proposals and on the development of new scientific leads from existing programmes. The committee, however, agrees with Lord Zuckerman that "a steady and substantial increase over the years" is likely to yield valuable results.

Senior Posts

The committee has already welcomed the decision of the Cancer Research Campaign to establish a number of new chairs of clinical oncology. It also takes the view that there is a need for the establishment of further consultant posts within the National Health Service for specialists in the cancer field. Some of these posts will undoubtedly be needed both where there are chairs of clinical oncology and in oncological centres. The committee also considers that some might be associated with major hospitals, especially teaching hospitals, throughout the country. In the first two years it might be appropriate to establish ten such new consultant posts. While initially many of these posts will be filled by clinicians trained in relevant specialities that are currently recognized within the Health Service, the committee hopes that in the longer term the necessary steps will be taken to establish oncology as a speciality in its own right, together with its own career structure.

There is also a need to establish a number of additional senior non-clinical posts with security of tenure. The committee envisages that in addition to being held in existing cancer research institutes, oncological centres, university departments and other institutes undertaking relevant basic research, a number of such posts should be tenable in clinical departments (including those in universities) where no career structure exists at present for those wishing to remain in a clinical environment. The committee believes that there is scope for the establishment of between eight and ten new posts of this kind at about senior lecturer level within the next two years.

Training Posts

The committee recommends that in the immediate future between twenty and thirty additional training posts should be provided for work in each of the disciplines already identified as being of particular relevance to cancer, that is, immunology, virology, epidemiology, developmental biology, mammalian cell genetics, and also in radiobiology. The majority of these posts might be held in university departments, but some should be in the cancer research institutes as well as in basic research institutes with training facilities such as the National Institute for Medical Research.

There is an urgent need to attract clinicians to specialize in oncology and the committee suggests that in the longer term it may be necessary to establish additional training posts at the registrar or senior registrar level within the National Health Service for this purpose. The consultant posts and chairs in oncology mentioned above would ensure that such doctors were entering a speciality with career prospects comparable to those in other disciplines. The number of training posts needed would depend on the number of consultant posts actually established and planned, and the number and quality of recruits coming forward to fill them.

Travelling fellowships are also needed for more senior workers and the committee recommends that five such fellowships should be allocated annually for this purpose.

If these proposals are accepted, there will be a need to finance supporting staff—technical staff, social workers, statistical and secretarial staff and so on.

Multi-centre Clinical Trials

The committee wishes to emphasize the importance of organizing more multi-centre trials, many of which will require a full-time medically qualified coordinator, and clerical and other assistance. In view of the rapid increase in the introduction of new methods of treatment it is recommended that four or five tenure posts should be established for this purpose together with supporting staff.

Clinical trials are useful not only in determining the effectiveness of new forms of treatment, for example, asparaginase and immunotherapy in leukaemia, but also in assessing the comparative benefits of combinations of different forms of therapy, for example surgery and radiotherapy. It is often necessary to conduct such trials on a multi-centre basis, because individual centres seldom have enough comparable cases within a short enough period. Although clinical trials may demonstrate only very marginal advantages of one form of treatment over another, they provide a certain way of making advances in the treatment of cancer and are also valuable when they demonstrate that a complex form of treatment which puts the patient to great inconvenience is unnecessary.

Screening Facilities

The committee envisages a need for the early commitment of funds for further research on the benefits of mass screening techniques. Pilot studies are not at the moment sufficiently far advanced for it to be possible to be precise in making recommendations, and any such projects would need to be discussed in detail with the Health Departments.

In general it may be said that the earlier the stage at which cancer is diagnosed the more amenable it will be to treatment, and the greater the chance of a clinical cure. In some instances special techniques can be used to demonstrate early malignant change and these methods are applied to a "normal" population in mass screening. To assess the potential contribution of such techniques, information has to be obtained on their sensitivity, specificity and acceptability, and large numbers of people may need to be examined and kept under observation for several years.

Facilities

The committee welcomes the decision to set up four new oncological centres in England on a trial basis. The Scottish Home and Health Department is currently considering the organization of oncological services in Scotland, in the light of a report received from the Standing Cancer Committee of the Scottish Health Services Council. This report advocates the development of the Beatson Institute for Cancer Research as the single Cancer Research Institute in Scotland and, if accepted, will undoubtedly lead to a requirement for further accommodation. Plans for an oncological centre in Wales, based in Cardiff, are under consideration by the Welsh Office.

The committee hopes that it will be possible to establish new research teams in these centres and recommends that funds should be earmarked for this purpose. If full advantage is to be taken of this opportunity to foster close liaison between clinical and basic research teams it will be essential to provide sufficient funds for the development of first-class facilities for patient care in these centres.

The committee strongly endorses Lord Zuckerman's view that accommodation for research could be improved. It has, in particular, identified the following as being likely to need an early injection of funds:

The Institute of Cancer Research exists at present on three widely separated sites (Fulham Road ; Sutton, Surrey ; and Chalfont St Peter, Bucks). There is an urgent need to rebuild the institute on one site at Sutton, Surrey. Plans are in hand which await only the necessary funds.

The Beatson Institute for Cancer Research in Glasgow. Plans for rebuilding the institute are far advanced, but although a grant of £360,000 has been awarded by the Wolfson Foundation a shortfall of about £100,000 in the total cost is anticipated during the next two years. A substantial further injection of funds will be required in the longer term.

Other opportunities for further expenditure include the support of a clinical unit associated with the new Tumour Biology Group at the MRC Laboratory of Molecular Biology at Cambridge ; the development of facilities for trials of neutron therapy ; the rehousing of the Cancer Epidemiology Unit at Oxford, now inadequately accommodated in three separate buildings ; the provision of a new building for the MRC Mammalian Genome Unit at Edinburgh ; the staffing and equipping of an experimental unit to work in parallel with the clinical research unit established by the ICRF at St Bartholomew's Hospital ; and the rebuilding and modernization of the ICRF Laboratories at Mill Hill.

The committee is also investigating the possibility that the Microbial Research Establishment at Porton might do more work in relation to cancer.

Expanded Cancer Research Programme

The implementation of the specific proposals above will depend in the case of clinical and laboratory workers on the number of applicants of the requisite potential who in the event come forward. The committee believes that it should be possible to fill the posts specified within, say, two years of funds being made available. Some of the specific areas of research and projects suggested above are already under consideration by the sponsoring bodies. Others would need to be most thoroughly investigated before a final commitment was made. In many cases rapid decisions could be made, but in others preliminary work on the precise definition of the scientific programme, the recruitment of staff and provision of facilities would be required. Now, as always, when there is no single obviously correct path to follow, it will be important to ensure flexibility and to allow for the rapid switching of emphasis to take advantage of new leads. The committee accordingly recommends that a contingency fund of £100,000 should be made available annually as an uncommitted pool. Thus the committee has tried to put figures to its proposals and to indicate the possible shortfall between the present commitment of private and public funds, and what could be earmarked if it were desired to accelerate the pace of cancer research still further in Britain.

Table 1 Estimated Annual Costs

	Annual cost (£)
3 chairs of clinical oncology	81,000
10 NHS consultant posts	90,000
8–10 senior non-clinical posts	154,000
20–30 non-clinical training potss	80,000
6 clinical training posts	27,000
5 travelling fellowships	25,000
4–5 staff for clinical trials	25,000
Contingency fund	100,000
	582,000

The committee wishes to re-emphasize that any expenditure of funds on cancer research must continue to be the subject of the most stringent and uniform scientific scrutiny in order to ensure that the quality of work is maintained at the highest standard.

The costs of a development programme along these lines are estimated in the accompanying tables. Table 1 shows the estimated annual cost of the opportunities listed above, and consists chiefly of the direct cost of salaries and staff support. It is important that the proposal for the establishment of chairs in clinical oncology is likely to be contained in the present plans of the Cancer Research Campaign. Much of the annual contingency fund would be needed by research teams associated with the oncological centres.

Table 2 Estimated Capital Costs

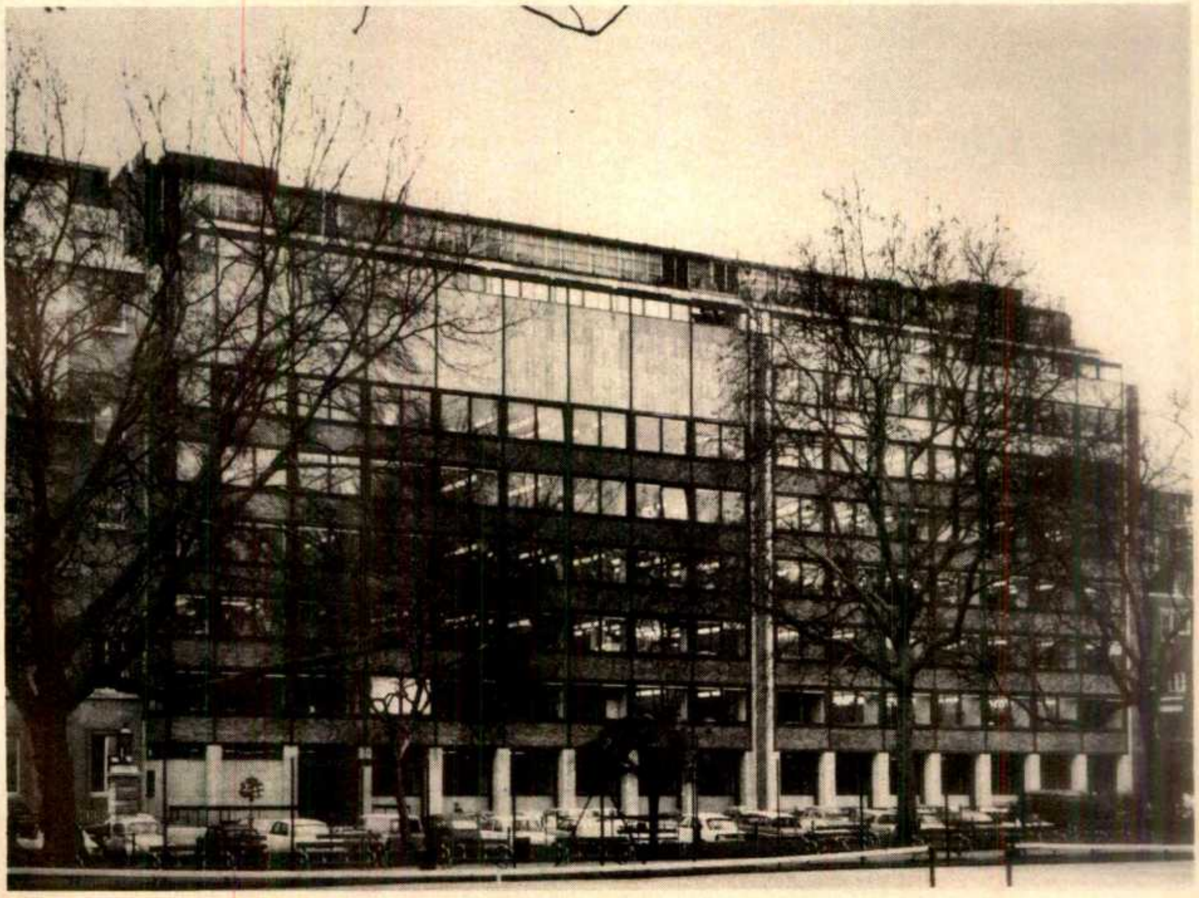
	Cost (£ million)
Institute of Cancer Research	3.50
Beatson Institute	0.80
ICRF, Mill Hill	1.00
Oncological centres	1.00
ICRF, St Bartholomew's	0.50
Clinical Tumour Biology Unit	0.80
Neutron therapy	1.00
Epidemiology Unit	0.08
Mammalian Genome Unit	0.22
	8.90

Provision for service aspects of oncological centres, family care, education and possible developments at MRE, Porton, is not included.

Table 2 shows the estimated capital cost of some of the development schemes listed above. Of the total capital cost of £8.9 million, £3.7 million would be needed in the first two years of an accelerated programme. Not all the cost would fall on the government—private sources, for example, are likely to contribute £250,000 to the rehousing of the Institute of Cancer Research, while the cost of developments such as the MRC Clinical Tumour Biology Unit is already included in the budgets of the departments concerned. Allowing for arrangements of this kind already made, the total extra capital cost is likely to be of the order of £1.7 million in the first two years of an expanded programme.

Making similar allowances, £501,000 is the committee's best estimate of the maximum level of annual recurrent expenditure that might be reached towards the end of a two year period. There would be a gradual build-up to and beyond this level and the proportion that might be spent within the two years might be about £600,000. This figure, together with that of £1.7 million non-recurrent for building and facilities, is therefore the committee's best estimate of monies which if committed now might reasonably be expected to be usefully spent within the next two years on an expanded and accelerating national cancer research effort. It must, however, be emphasized that as far as recurrent expenditure is concerned the commitment should be regarded as long term, and that, in the case of capital expenditure, the greater part will be incurred after the initial two year period. The sums suggested will need to be reviewed annually and adjusted—almost certainly upwards—as programmes develop.

Earlier in this article the committee emphasized the point made in its first report that it was important that cancer research should not be carried out in isolation from the main stream of other biomedical research. Lord Zuckerman recognizes in his report that one of the effects of the American initiative will be to increase the pace of biomedical research. He has also commented that while fundamental research in this country may not hitherto have suffered from lack of funds, this situation may change in the near future. The committee agrees with these assessments. It thinks that it is of great importance to cancer research that there should be no diminution in the scale of the basic biomedical research effort already going on in Britain, and, in particular, that none of the proposals that they have made above should be implemented at the expense of other areas of biomedical research.



The Imperial Cancer Research Fund building in Lincoln's Inn Fields, London.

Palaeohydrology of Late Pleistocene Lake, Alexandersfontein, Kimberley, South Africa

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The closed Alexandersfontein depression, now only an evaporation pan, harboured a +19 m lake with a surface area of 44 km^2 and Middle Stone Age occupance, a little before 16,000 BP. Assuming a temperature depression of 6° C, calculations show that rainfall was about twice that of today.

CONTROVERSY prevails as to the nature of Pleistocene climates in the interior of southern Africa[1-4]. For example, it is uncertain whether geomorphological[5] and palynological[6,7] indicators of relatively moist late Pleistocene climatic episodes reflect lower temperatures, higher rainfall, or both. In order to reduce some of the multiple, interacting factors that generally impede interpretation, a closed depression east of Kimberley was studied in 1971. Here a 328 km^2 basin drains ephemerally into the Alexandersfontein Pan, which lies athwart the Cape Province-Orange Free State border at approximately 28° 50′ S and 24° 48′ E. The floor of the pan receives a little spring influx but now only holds water sporadically after heavy rains; however, the depression once enclosed a number of substantial Pleistocene lakes.

The Alexandersfontein basin is formed on subhorizontal Dwyka shales extensively intruded by Karroo "dolerite" (diabase) sills, dykes and plugs that provide koppies of relief 30 to 100 m. Total elevation ranges from 1,119 m on the pan floor to 1,270 m along the irregular rim of the watershed. Whatever factors may have initiated the depression, one of countless such features in erosional topography around Kimberley[8-10], Pleistocene and later excavation must be attributed to periodic deflation, with crucial chemical weathering of the shales to which deepening is confined. The oldest surficial deposits are fragmentarily preserved, with little or no primary relief, along the eastern, southern and western margins of the depression (see Fig. 1). These consist of lacustrine and aeolian beds, often reworked, and reaching 55 to 60 m above the modern pan floor, although the present threshold of the basin is clearly defined at 1,151 m (+32 m), forming a conspicuous former overflow outlet to the Modder River drainage.

The younger lake beds under consideration here are shown in Fig. 1. They are better preserved and extensively exposed under a veneer of wash and drifting sand, and they seem to be confined by the 1,142 m (3,800 foot) contour of the 1:50,000 topographic maps (Nos. 2824 DB/DC/DD). Beach ridges of these younger lakes are well developed south of the modern pan (Fig. 1), with shoreline cliffs locally cut into shale along the northern peripheries. Specific stages are recorded at +17 to 19 m, +12 m and +6 m (see Fig. *2a* and *b*).

Along section A (Fig. *2a*), the highest lake beds reach +19 m and consist of a white, friable, "chalk": 81.5% CaCO$_3$, 3.5% opaline silica, and a further residue of silty clay. A sample of inorganic carbonate from a depth of 1 m gave a date of 16,010±185 BP, with ^{13}C = −0.77‰ (SI-1115). As there is no carbonate bedrock in the basin, except for localized surface calcretes, this age must be considered a minimum, although the silica structure, which does not break down in HCl, has presumably restricted recycling of the carbonates. The +12 m beds form more conspicuous ridges here, locally with a 2 m nip. One section (A, Fig. *2a*) begins with a prismatic, brownish-grey, sandy silt (14.5% CaCO$_3$), which followed by a columnar, pale-brown silty sand (27% CaCO$_3$), by weakly stratified, yellowish-brown sandy clay (6% CaCO$_3$), and ultimately a coarse, reddish wash. Whereas the sands of the three lower beds are derived from breakdown of local shales and diabase, the final wash includes subrounded, exotic quartz of aeolian type. Forms and deposits show that peripheral fluvial activity accompanied the +12 m lake stand, with the basal lacustrine beds succeeded by a lake-margin alluvium, and finally a fill, now exposed as a dissected fan.

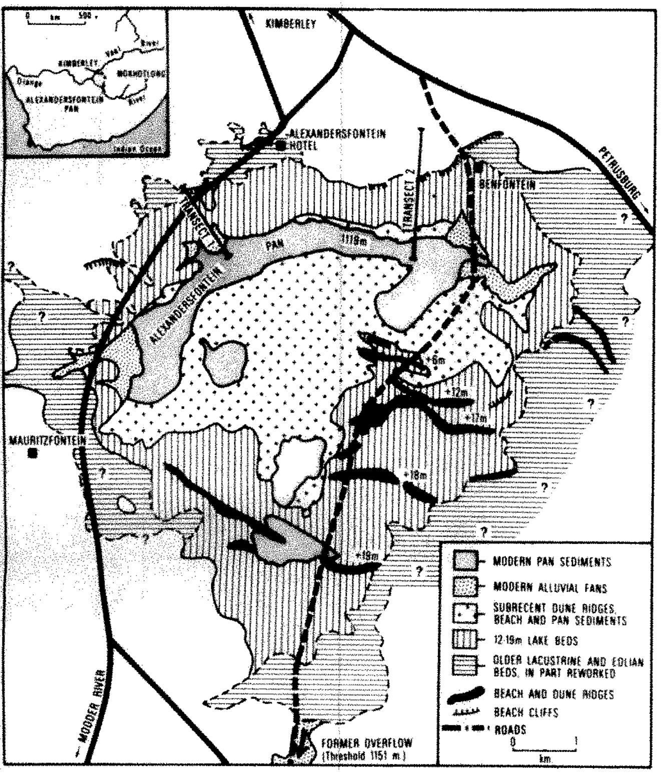

Fig. 1 Surficial deposits of the Alexandersfontein Pan.

Along section B (Fig. 2*b*) the +19 m lake is recorded by the upper half of a sequence that begins with a wash of silty sand ($CaCO_3$ sharply increasing from 7 to 27% at the top). Then follows a prismatic, light-grey marl (79% $CaCO_3$, 6% amorphous silica) with a silty clay residue. The abundant small gastropods *Pupilla* (*Gibbulinopsis*) *fontana* (Küster), *Pupilla* (*Afripupilla*) *tetrodus* (O. Boettger), *Vertigo antivertigo* (Draparnaud) and *Succinea striata* (Krauss) are terrestrial, but the last two are hygrophile, and we infer that these snails were reworked by rainwash or after a seasonal rise of lake level. A bulk sample of essentially inorganic carbonate gave a date of 11,025±110 BP (SI-1116), with $^{13}C = -0.44\%$. This is also a minimum date, but the secondary calcification and the presence of illuvial soil wash in small veins and hollows suggests a considerably greater degree of contamination by younger carbonates than in the case of SI-1115. These quiet-water, shoreline deposits are followed by a prismatic, light-grey clayey sand (9% $CaCO_3$ and 16% amorphous silica), and finally a similar silty sand (17% $CaCO_3$, 20% opaline silica). Along an alternate section (see Fig. 2*b*), the final bed is overlain by 2 m of well-stratified, gritty alluvium that was truncated before a prismatic, dark-grey clay was laid down. These additional details suggest a temporary regression, accelerated runoff and alluvial influx, culminating in a transgression that cliffed the deposit, with the dark cracking clays probably laid down on the lake margin a little later. The sands are again local except for some rounded, exotic quartz in the penultimate bed, preceding the temporary regression.

Altogether the +17 to 19 m lake (with beach ridges or cliff bases at those levels, but undifferentiated deposits to +23 m or higher) represents a long interval with appreciable oscillations of the shoreline, paralleled by persistent peripheral runoff and changes in the level of aeolian activity. The differential degree of "silicification" suggests a moderate to long time span before development of the +12 m shoreline, under similar conditions, with a last halt in regression at +6 m. Extensive deflation then attacked the centre of the basin, creating four separate pans of which the Alexandersfontein is by far the largest. Recent sedimentation involves a cracking, greyish-brown clayey silt, with 17 to 25% $CaCO_3$, a trace of amorphous silica, and sands entirely of local origin (shale residues, feldspar, trace quartz); depending on the local importance of sodium salts, *p*H varies from 7.9 to 9.0. Other recent or sub-recent deposits (Fig. 1) include extensive, stepped aeolian beach ridges or lunettes, with traces of higher lake "muds" to +3 m (see Fig. 2*b*).

As two ^{14}C dates of a little over 17,000 yr were obtained from large aquatic snails in terminal high-floodplain deposits of the Vaal River northeast of Kimberley[11], we feel that the +17 to 19 m lake very probably has a date of about 16,000 BP or a few millenia before. It is beyond question contemporary with the maximum glacial advance in higher latitudes, in what has been designated the Würm Upper Pleniglacial[12], and *Vertigo* is a Palaearctic form. No materials suitable for dating have yet been recovered from the +12 m lake beds, but a Late Glacial age may apply here.

This is the type area where J. H. Powers collected the "Alexandersfontein variant" of the Middle Stone Age (MSA)[13], and we found similar artefacts resting in profusion on the dark clays along transect *b* (Fig. 2), while Powers's collections came from wash on denuded surfaces of the +19 m lake beds or shorelines northeast of transect *a* (Fig. 2). A single, convergently-patterned, large Levallois flake on lydianite was found *in situ* well within a calcrete mantling the +12 m lake beds along transect *a*. Local MSA settlement was almost certainly coeval with the last stages of the +17 to 19 m lake, and may possibly have extended until (or resumed with) the +12 m lake. Corroboratively, D. M. Helgren and Leon Jacobson (personal communication) have recently discovered an MSA site with mammalian fauna *in situ* and at least partly in primary context in the 18 to 19 m shoreline south of the lake.

The palaeoclimatic implications of high, non-outlet lakes in the Alexandersfontein depression are considerable. The +17 to 19 m lake had an area of 44 km^2 and an average depth of almost 8 m, whereas the +12 m lake had an area of 24 km^2 and an average depth of 4 to 5 m. Evaporation over open water (by a Symons pan) now averages 2,120 mm yr^{-1} in Kimberley[14], whereas precipitation (averaged for nine stations in the quadrant 28° 50′–29° 00′ S, 24° 45′–25° 00′ E) is only 397 mm (ref. 15) with a calculated potential evapotranspiration of 932 mm, precluding all but incidental runoff from the drainage basin. In the upper Vaal drainage with almost double the rainfall and temperatures 2 to 3° C lower, the runoff quotient is only 9.3% or about 59 mm (ref. 16), implying that the calculated evapotranspiration by the Thornthwaite method here is 17.7% too high[17]. In the case of the +12 m lake, 1 mm of runoff across the entire basin would provide an influx equivalent to 11.7 mm of water over the 24 km^2 of lake surface, and in the case of the +19 m lake, 6.4 mm over a 44 km^2 lake. Consequently, assuming constant evaporation factors and no external groundwater losses or gains, and a reduction of potential evapotranspiration by 17.7%, the +12 m lake level could be maintained only with a precipitation of 1,046 mm and a runoff ratio of 0.9%.

It would be more realistic to assume a temperature depression of 6° C, a conservative estimate for the Würm Pleniglacial on the basis of periglacial and other cold-climate phenomena in South Africa[18–20]. Recalculating the potential evapotranspiration, this gives 695 mm for Kimberley, which is identical to that in the upper Vaal drainage today[17], so that the 9.3% runoff quotient can be safely used. Actual evaporation from open water exposed to a reduction in temperature of 6° C would be

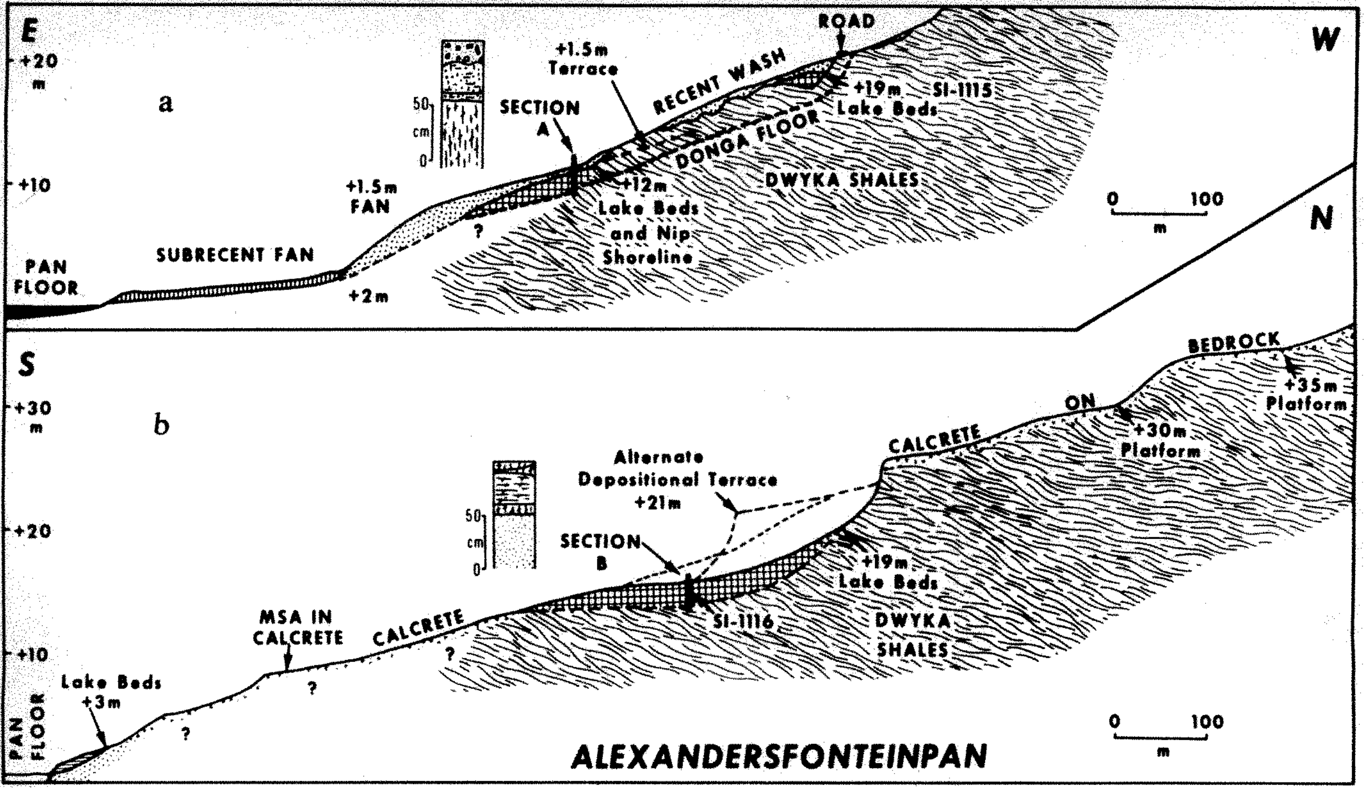

Fig. 2 *a* and *b*, Scale transects north of the Alexandersfontein Pan.

approximately 1,400 mm by analogy from the modern station Mokhotlong, Lesotho (12° C mean annual temperature compared with 18° C for modern Kimberley)[21]. In this case the hydrological budget would require 878 mm to balance with a +19 m lake and 671 mm in the case of the +12 m lake. By implication, with a 6° C drop of temperature, rainfall would theoretically need to be increased by 123% to maintain Pleniglacial Palaeolake Alexandersfontein at +19 m. The assumptions necessary to derive this figure caution against its use in more than a semi-quantitative manner. Even if temperatures and radiation received were substantially lower, however, rainfall would necessarily have been almost twice that of today.

Applying these data to a monthly lake budget, assuming a seasonal precipitation and evaporation regime analogous to that of today, and determining relative monthly distribution of runoff by a calibrated version of the Thornthwaite-Mather hydrological budget[22], a seasonal fluctuation of 4.1 cm could be inferred for the +19 m lake, with a maximum level in May and a minimum in December. Such computations are, however, unrealistic, for it is necessary to assume seasonal changes of rainfall and radiation.

Nonetheless, the Pleniglacial climate of the Kimberley district was necessarily both substantially cooler and wetter than today—in a relative as well as absolute sense—so corroborating van Zinderen Bakker's interpretation of the Florisbad pollen profile[6] and Coetzee's of that at Aliwal North[7]. This demonstrated wet period in the Interior Plateau of South Africa coincides with high lake levels in the middle latitudes but contrasts with a marked Pleniglacial dry phase in many parts of tropical Africa[23]. Two meteorological interpretations are possible. Equatorial displacement of the zonal boundary between the southern circumpolar westerlies and tropical easterlies during a Pleniglacial circulation[24] would significantly increase winter precipitation, decrease summer rainfall. On the other hand, the northern hemisphere circulation may have been appreciably strengthened during the Pleniglacial, displacing the tropical circulations of Africa southwards and thereby bringing the Kimberley region well within the summer rainfall belt (J. Bjerknes, personal communication).

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Effects of Lysozyme on Normal and Transformed Mammalian Cells

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Homologous mammalian lysozymes have specific effects on the *in vitro* growth patterns and morphology of normal and transformed mammalian cells, apparently due to effects on constituent membranes. Lysozyme may be important in the surveillance of membranes and may be a mediator of the antitumour functions of macrophages.

IN 1922, Fleming[1] reported a "remarkable bacteriolytic element found in tissues and secretions", which he designated "lysozyme" (LZM)—an enzyme capable of lysing (bacteria). Fleming's studies and other extensive investigations in the ensuing fifty years (see ref. 2 for a recent review) documented the widespread distribution of LZMs in animal and plant tissues, elucidated their detailed structures, particularly those of hen egg white and human origin, and defined the precise mechanism of LZM's interaction with and cleavage of polysaccharides, chiefly those known to be constituents of bacterial cell walls. Fleming's concentration on the bacteriolytic actions of LZM and its role in antibacterial defence mechanisms probably limited speculation and investigation of possible non-antibacterial actions of LZM which may be of equal if not greater importance in mammalian physiology.

We have investigated the possibility that certain polysaccharides, glycoproteins or glycolipids of mammalian cells are susceptible to enzymatic cleavage by LZM. As these substances are major constituents of cell membranes, we further proposed that LZM may be important in the regulation of certain membrane-dependent cell functions and may participate in the surveillance of membrane abnormalities, particularly those associated with neoplastic transformation.

We examined the effects of LZM on the behaviour of normal and transformed murine and human cells *in vitro*, thereby seeking insight into the possible effects of endogenous LZM *in vivo*. We used homologous mammalian LZMs, that is LZM isolated from the urine of patients with monocytic

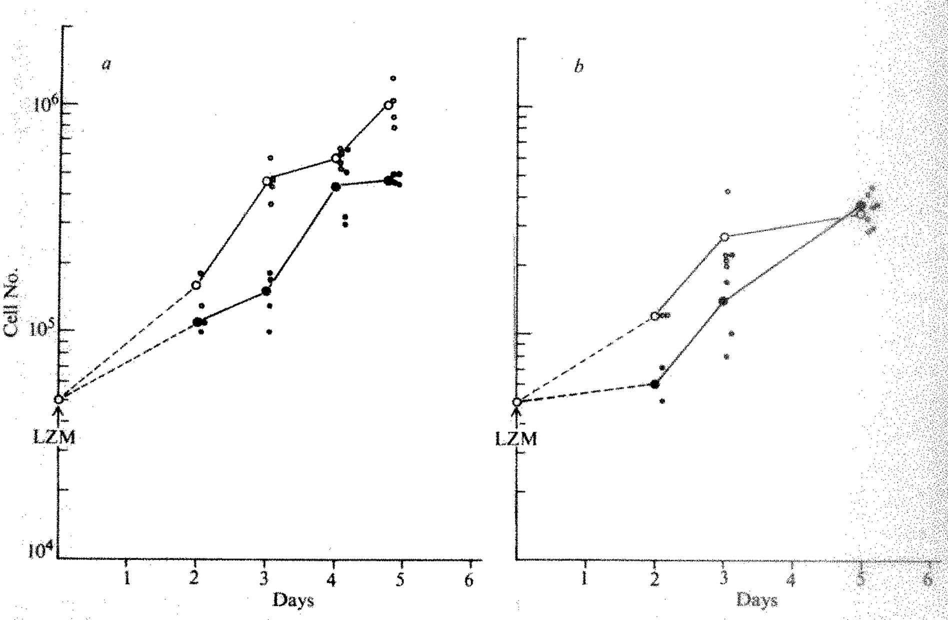

Fig. 1 Effect of rat LZM (100 μg ml^{-1}) on the proliferation of (*a*) normal (3T3) and (*b*) transformed (SV101-3T3) mouse embryo fibroblasts. The initial cell inoculum was 5×10^4 cells in a 5 cm diameter (Falcon plastic) culture plate, and LZM was present in the medium throughout the entire period of culture. ○ and •, Individual plate counts; ○ and ●, mean of each group. Black symbols, LZM; open symbols, control.

leukaemia[3] and of rats bearing the transplantable chloroleukaemia which elaborates LZM (ref. 4). Available evidence indicates that these LZMs are identical to their normal counterparts[3]. LZMs were isolated from urine by the bentonite adsorption method[3]. The purity of all preparations was ascertained by appropriate electrophoretic, chromatographic and immunological analyses.

Mouse Embryo Fibroblasts

Normal mouse embryo fibroblasts (3T3) and simian virus-transformed cells (supplied by Dr Claudio Basillico, New York University School of Medicine) were grown at 37° C in a 7% CO_2 : air atmosphere in Dulbecco's modified Eagle's medium (MEM) supplemented with 10% calf serum, penicillin (100 U ml^{-1}) and streptomycin (50 U ml^{-1}). In the experiments shown in Fig. 1, rat LZM (100 μg ml^{-1}) was added to the cultures at the time of initial plating. The initial cell inoculum was 5×10^4 cells in a 5 cm diameter culture plate. In these conditions of relatively low cell density and the presence of LZM throughout the culture period, the LZM appeared to effect a transient inhibition of the proliferation of both 3T3 and SV-3T3 cells, chiefly evident during the first 4 d in culture. At the fifth day, particularly with the SV-3T3 cells, there was no significant difference in the total cell numbers in the control and LZM-treated cultures. As Fig. 2 shows, however, there was a significant qualitative difference between the control and LZM-treated SV-3T3 cultures on the fifth day. Thus, in the control SV-3T3 cultures there was dense growth in the middle of the plate and sparse growth at the periphery, whereas the LZM-treated SV-3T3 cells were more uniformly distributed over the surface of the culture plate. Both the control and LZM-treated 3T3 cells were relatively uniformly distributed in their growth patterns. When rat LZM (100 μg ml^{-1}) was added to 3T3 and SV-3T3 cultures 48 h after the initial plating of 1×10^5 cells, that is twice the inoculum as in the study shown in Fig. 1, there was much less of an effect than in the previous experiments.

Fig. 3 shows the effects of increasing concentrations of rat LZM present from the time of initial plating on the uptake of ^{3}H-thymidine by normal (3T3) and transformed (SV-3T3) mouse embryo fibroblasts cultured on hanging glass coverslips[5]. In both cell lines LZM produced a small but significant and apparently dose-dependent decrease in uptake. We also noted, particularly with the 3T3 cultures, that the LZM inhibition of ^{3}H-thymidine uptake was most evident in the first 2 d *in vitro*, similar to the pattern observed in the cell counting studies.

Transformed Rat Liver Cells

Because LZM might alter different cells in different ways, we tested the effects of rat LZM on transformed rat liver cells. A cell line (RLB) which had apparently undergone spontaneous transformation when grown in a nutritionally-deficient medium was obtained from Dr Carmia Borek of the College of Physicians and Surgeons of Columbia University[6]. This line was maintained in RPMI 1640 medium supplemented with 5% foetal calf serum, penicillin (100 U ml^{-1}) and streptomycin (50 U ml^{-1}). The effects of rat LZM in concentrations up to 400 μg ml^{-1} on the uptake of ^{3}H-thymidine by these cells were essentially comparable with those seen with 3T3 and SV-3T3 cells. Some inhibition was evident during the first 2 d in culture, but by the third day there was no difference between the control and LZM-treated cells with respect to ^{3}H-thymidine uptake. There were, however, significant cytological differences between the control and LZM-treated RLB cultures which were already evident after 2 d and were more marked after 4 d (Fig. 4). Whereas the control cultures exhibited a relatively uniform, epithelial hexagonal pattern, the LZM-treated cells showed very marked nuclear and cytoplasmic irregularities. Thus, the nuclei of the LZM-treated cells varied considerably in size, shape and staining intensity, and many appeared distinctly pyknotic. The cytoplasmic changes in the LZM-treated cells consisted of marked spreading, development of surface invaginations and the extension of long, filamentous processes. We also noted that the control cells contained numerous clear droplets, presumably lipid, which were relatively uniform in size and mostly perinuclear in distribution, whereas the LZM-treated cells contained fewer droplets which were more irregular and scattered throughout the cytoplasm.

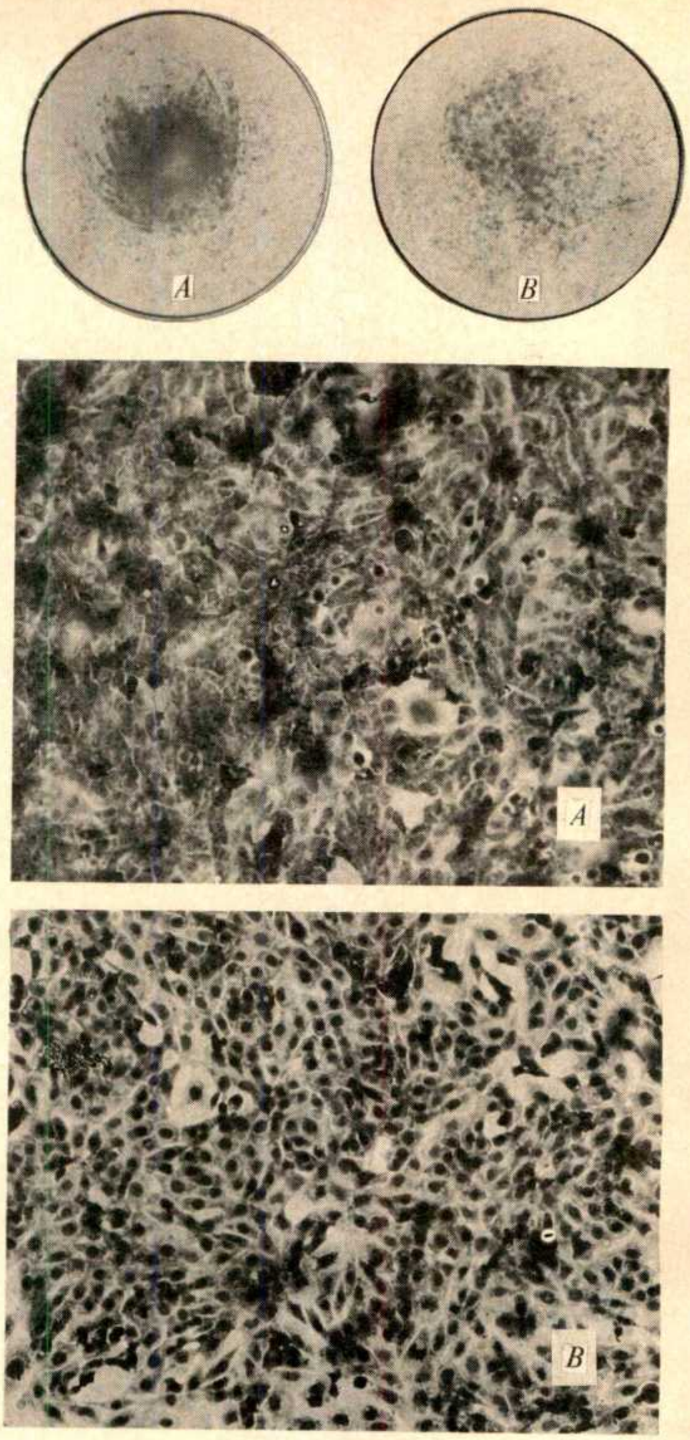

Fig. 2 Gross and microscopic appearance of SV101-3T3 cultures; (*A*) control and (*B*) grown in the presence of rat LZM 100 μg ml^{-1}, on day 5 of the experiment shown in Fig. 1. The LZM-treated culture shows a more uniform distribution on the plate and a more regular cytology with less pleomorphism.

Although some of the cytological changes associated with LZM treatment of these cells might suggest cell damage and degeneration as a consequence of a non-specific toxic effect of LZM, the ^{3}H-thymidine incorporation data indicate that the net synthesis of DNA in these cultures was not markedly reduced by LZM.

Electron Microscopy

To document further the effects of LZM on the surfaces and organelles of parenchymal (hepatic) cells, scanning (SEM) and transmission electron microscopic (TEM) studies of human cell cultures were carried out (in collaboration with Professor Marcel Bessis and Dr Yves Le Charpentier at the Institute of Cellular Pathology, Paris (Bicêtre)). The cell line used was originally derived from a liver biopsy of a 4-yr-old girl with haemolytic anaemia and microspherocytosis. The tissue culture was started in January 1972 and showed restricted growth for 2 months (four passages) at which time it apparently transformed spontaneously and proliferated more rapidly. At the time of our study (August 1972), the cells had been passaged fifty times. They were grown on glass coverslips in Leighton tubes using Eagle's medium supplemented with 20% foetal calf serum, glutamine, vitamins, heparin and antibiotics. After dehydration, the coverslips were air dried ("critical point" method) and the cells coated with a conducting layer of vacuum evaporated gold. The coverslips were attached directly to the specimen holder of the microscope (Cambridge–Stereoscan, operated at 30 kV). Pictures were taken at ×500–6,000 at about 40° to the monolayer surface. Parallel coverslip cultures were prepared and processed for transmission electron microscopy (Philips EM 200).

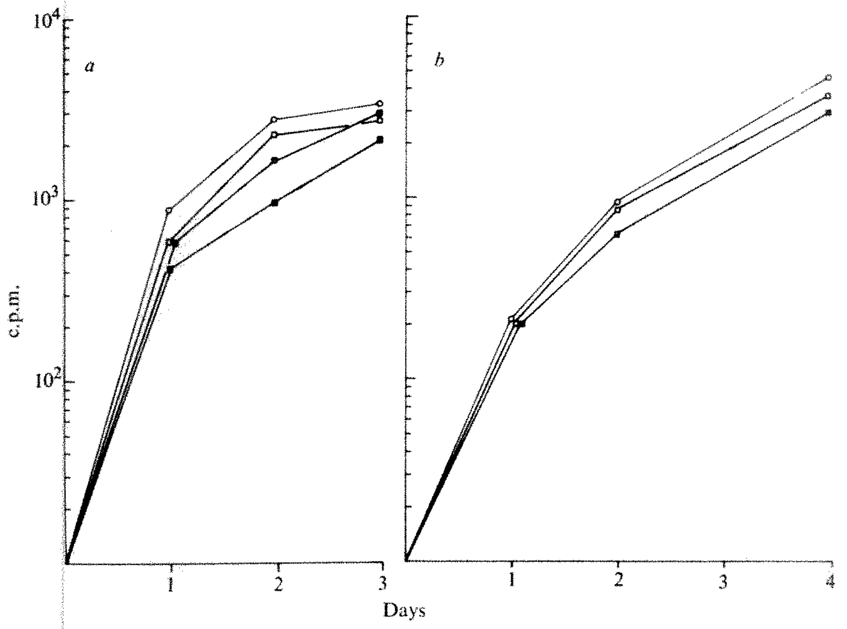

Fig. 3 Effects of increasing concentrations of rat LZM (200 to 800 μg ml^{-1}) on the uptake of ^{3}H-thymidine by (*a*) normal (3T3) and (*b*) transformed (SV101-3T3) mouse embryo fibroblasts. Cells were grown as monolayers on 11 × 22 mm hanging glass coverslips, according to the method described by Rytömaa[5]. The coverslips were initially placed along the down side of sterile plastic culture tubes (16 × 125 mm) in the horizontal position. The volume (0.5 ml) between the lower surface of the coverslip and the test tube wall was then filled with a suspension of cells (10^3 to 10^4 per ml) in tissue culture medium containing ^{3}H-thymidine (1 μCi ml^{-1}). After filling each tube, it was quickly rotated 180° along its horizontal axis bringing the coverslips to the top side, thus permitting the cells to settle on its surface. In this position the tubes were closed with loose fitting plastic caps and placed in the incubator. In a separate experiment, it was determined that the majority of 3T3 and SV-3T3 cells were adherent to the coverslip surface within 30 min. In all experiments, sufficient numbers of cultures were set up initially to permit collection of three or four cultures after 1, 2, 3 and 4 d of incubation. At these intervals, the coverslips were removed from the culture tubes, air dried, fixed in 95% ethanol and washed with distilled water. They were then placed in scintillation vials containing 10 ml of 'Aquasol' (New England Nuclear Corp) and counted in a Packard Tri-Carb liquid scintillation spectrometer. In previous studies[5], it was established that the channel ratios and energy spectra of ^{3}H-thymidine labelled bone marrow cells attached to coverslips were not significantly different from those of cells suspended directly in the scintillation fluid or from ^{3}H-thymidine in solution. The initial cell concentration was 10^4 per ml for the 3T3 cells and 4×10^3 per ml for the SV-3T3 cells. Each point represents the mean of three to four cultures. ○, Control; □, 200 μg ml^{-1} rat LZM; ▨, 400 μg ml^{-1} rat LZM; ■, 800 μg ml^{-1} rat LZM.

Twenty hours after plating in the presence of human LZM (200 μg ml^{-1}), both control and LZM-treated cells were firmly attached to the glass surface, and cell numbers were approximately the same (Fig. 5). Control cells (Figs 5*A* and *C*) were relatively more compact and spherical than the treated cells, and their cytoplasmic borders were sharply demarcated with only a few short, filamentous projections. In contrast, cells grown in the presence of LZM (Figs 5*B* and *D*) were markedly flattened and spread out on the glass surface, and their cytoplasmic borders had numerous long, filamentous projections. Also, the surfaces of the control cells appeared relatively smooth although a fine granularity was revealed by higher magnification (Fig. 5*C*), whereas the surfaces of the LZM-treated cells (Fig. 5*D*) had patches of verrucous nodularity plus scattered, smooth-surfaced nodules. Cultures of these human liver cells with rat LZM (200 μg ml^{-1}) showed similar effects although less marked.

For the TEM studies (Fig. 6), cells were transferred directly from the glass surfaces and embedded for sectioning. Consistent with the SEM observations, the control cells displayed relatively smooth cytoplasmic margins whereas the cytoplasmic margins of the LZM-treated cells showed numerous villous projections. Fig. 6 shows further that the control cells contained numerous large, fat droplets principally clustered around the nuclei and probably responsible for the fine surface granularity seen by SEM. These droplets were virtually absent from the LZM-treated cells. Furthermore, in the control cells the mitochondria and ribosomes were irregular and poorly-

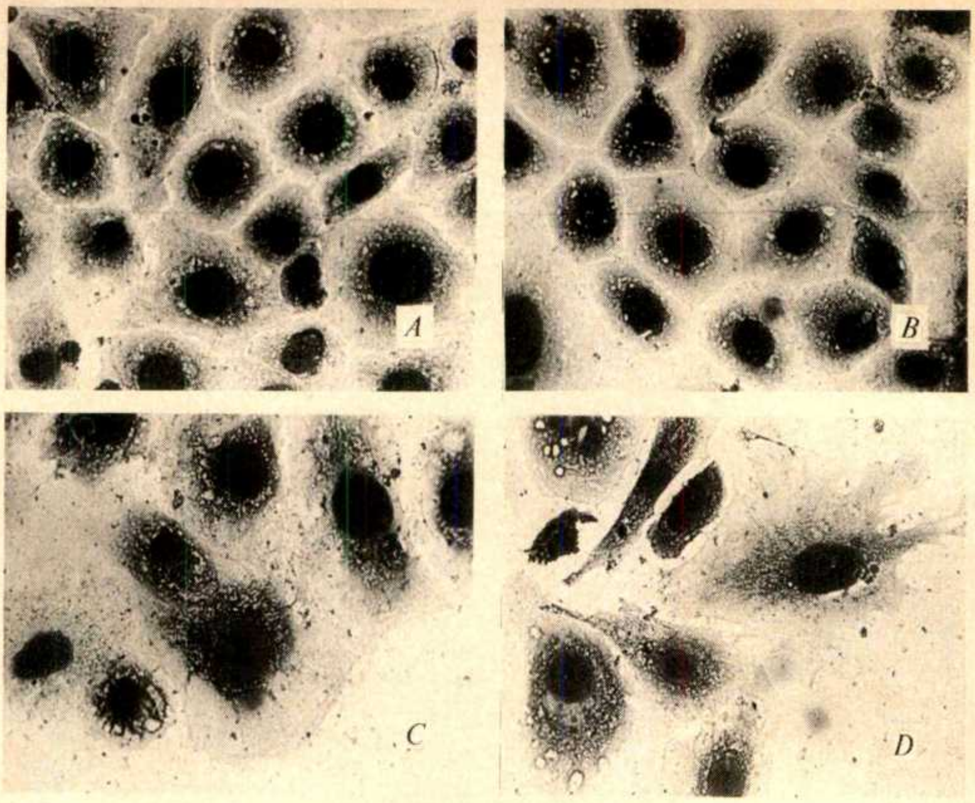

Fig. 4 Cytology of spontaneously transformed rat liver (RLB) cell cultures grown in control conditions and in the presence of rat LZM (400 µg ml^{-1}) for 2 and 4 d, respectively. After counting for ^{3}H-thymidine, the coverslips were washed in 95% ethanol to remove the scintillation fluid and then stained with Wright Giemsa stain for cytological examination. *A*, Control, 2 d; *B*, control, 4 d; *C*, rat LZM, 2 d; *D*, rat LZM, 4 d.

defined, whereas in the cells treated with LZM the mitochondrial membranes and cristae were more regular and sharply defined and the ribosomes more numerous and clustered in polysomes. Thus, with respect to certain features of internal structure, the LZM-treated cells appeared more normal than the untreated control cells. It also appeared that the outer ("plasma") membranes of the LZM-treated cells were thinner and more sharply defined than those of the control cells, consistent with a reduction in the amount of surface polysaccharide ("glycocalyx").

Implications

Our results indicate that LZM has significant effects on various mammalian cells *in vitro*, apparently not due to non-specific toxicity. The changes in cell configurations from globular to flattened contours and the development of fila-

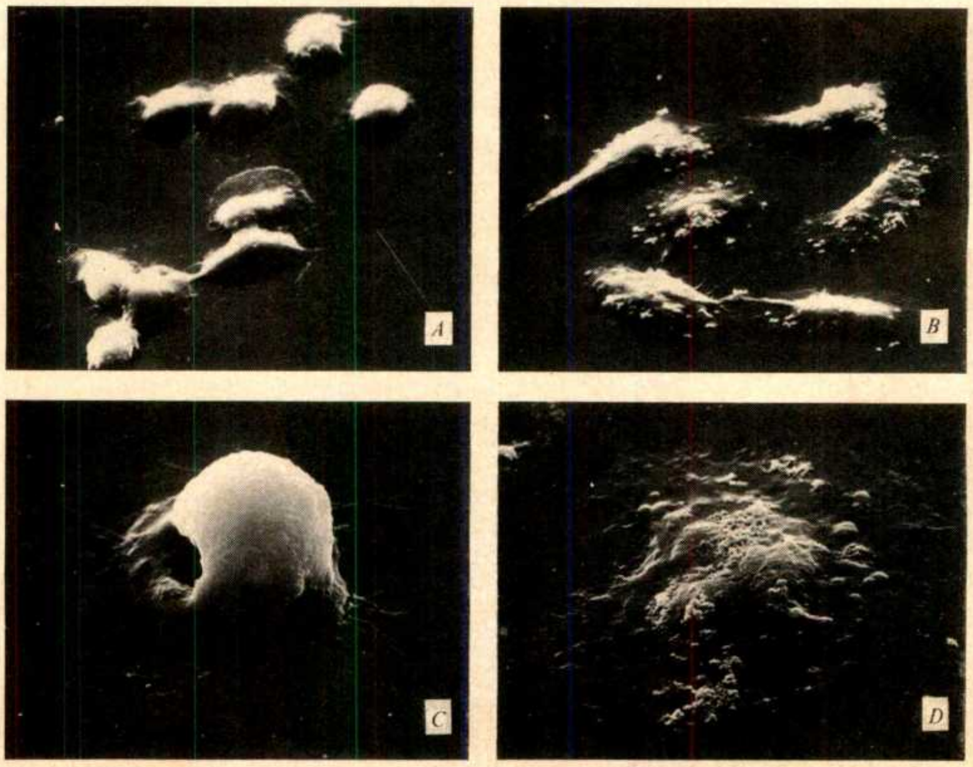

Fig. 5 Scanning electron micrographs of spontaneously transformed human liver cell cultures after 20 h *in vitro*. *A* and *C*, Control; *B* and *D*, human LZM (200 µg ml^{-1}). *A* and *B*, ×210; *C* and *D*, ×1,092.

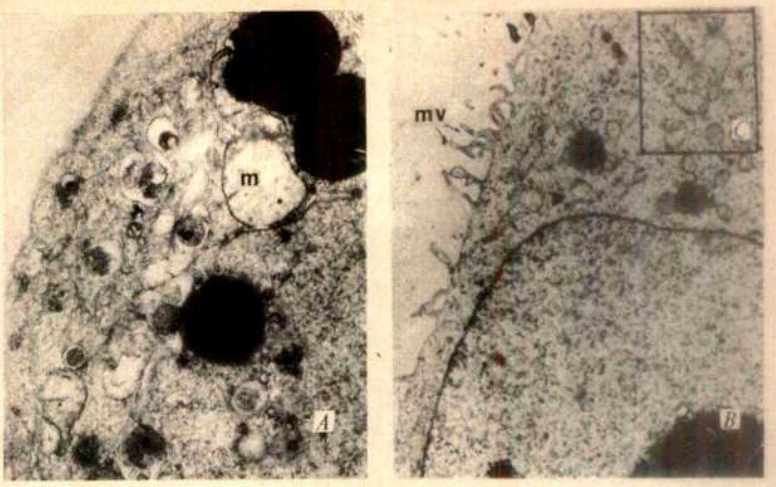

Fig. 6 Transmission electron micrographs of spontaneously transformed human liver cell cultures after 20 h *in vitro*. *A*, Control; *B*, human LZM (200 μg ml^{-1}); *C*, detail of (*B*) upper right. *A*, ×27,675; *B*, ×6,750; *C*, ×27,675. M, Mitochondrion; d, lipid droplet; mv, microvilli; r, ribosomes.

mentous cytoplasmic projections seen by electron microscopy imply a significant increase in total surface area, presumably accomplished either by extension ("stretching") of existing membrane or synthesis of new membrane, or both. The former could be due to the rearrangement of a lattice structure secondary to an effect of LZM on membrane polysaccharides or a non-specific polycation effect on the surface charge interactions with supporting surfaces[7–11]. Even if these changes are due to polycation effects, they may be significant when LZM is released from macrophages onto the surface of target cells. Alternatively or additionally, if LZM causes these cells to produce more membrane, the numerous mitochondria and polyribosomes and the reduced number of lipid droplets in treated cells may be related to this synthesis.

There is considerable, although mostly circumstantial, evidence that macrophages and histiocytes in tumours of man[12–17] and experimental animals[15,17] are important in host defences against neoplasia[15,16,18,19]. In addition, the antitumour effects of BCG vaccine and other adjuvants may be related to their ability to stimulate or activate macrophages[20–26]. The mechanisms and mediators involved are unknown, but LZM is elaborated by and released from macrophages[27,28], and increased production and/or release of LZM by macrophages has been demonstrated after BCG vaccination[28–30]. This information in conjunction with our findings suggests that LZM is a mediator of antitumour functions of macrophages. We also postulate that the cellular effects of LZM are due to a combination of its cationic properties, facilitating interaction with the negatively charged cell surfaces[7,8] and an enzymatic effect on (a) membrane polysaccharide(s). With respect to the first property, the polyanion heparin which strongly inhibits LZM activity against bacterial substrates[31,32] also inhibits the immune cell-mediated killing of mastocytoma cells[33].

We obtained our *in vitro* effects using homologous mammalian LZMs which can be isolated in gramme quantities from the urine of patients and rats with monocytic leukaemia[3,33], and which are apparently identical to their normal counterparts[3]. Further, the concentrations of LZM we used were probably within the range achieved *in vivo* at sites where macrophages interact with target cells.

Finally, we found relatively little *in vitro* toxicity of LZM, and some preliminary animal studies (unpublished results of E. F. O. *et al.*) indicate that relatively large amounts of homologous LZM (up to 5 mg g^{-1} body weight, administered i.v. or i.p., daily for several weeks) are well tolerated by mice and rats. Thus, if the biological usefulness of LZM is established, its pharmacological use is probably feasible.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Redshift of OQ172

WE report the discovery of a second QSO with a redshift greater than 3. Spectra taken at the 120-inch at Lick Observatory[1] give $z=3.53$ for OQ172. Carswell and Strittmatter[2] have found the redshift of OH471 to be $z=3.40$. Our attention was drawn to the object by the work of Gent *et al.*[3] on Molonglo radio sources. Accurate radio coordinates for OQ172 were communicated to us ahead of publication by C. Hazard and H. Gent. They are:

$$\alpha\ (1950)\ 14\ \mathrm{h}\ 42\ \mathrm{min}\ 50.48\ \mathrm{s}$$
$$\delta\ (1950)\ 10^\circ\ 11'\ 12.4''$$

A finding chart for OQ172 is given by Véron[4], who notes that it is a "blue stellar object". From our inspection of the Palomar Sky Survey plates we agree with Véron's assessment of the colour of OQ172, although Gent *et al.*[3] list it as stellar, that is an object with neutral colours. Radio fluxes for OQ172 are available from ref. 5, where the spectral index is given as -0.09 between 178 and 1,400 MHz. The tabulated fluxes at 1,400, 2,695 and 5,009 MHz are 2.42, 1.96 and 1.22 f.u., respectively[5]. The radio spectrum is therefore very flat and extends to high frequencies.

Figure 1 shows a spectrum of OQ172 obtained with the Cassegrain ITS system[6] at Lick Observatory. This spectrum is a composite formed by adding data from spectra obtained on different nights. Consequently the signal-to-noise ratio in the spectrum varies as a function of wavelength. It is particularly poor in the λ6000–λ6500 region where we have only one spectrum, to the red of λ7200 Å where OH emission features increase the radiation from the night sky, and shortward of 3500 Å where the atmospheric extinction becomes troublesome. In the spectral region between 4000 Å and 6000 Å the signal-to-noise ratio is good; most of the strong features present here are real and can be seen on the several individual spectra. The two strong emission features near λ5540 and λ7015 have been identified with Ly-α λ1216 and CIV λ1549. The measured wavelength of the emission peaks corresponds to a redshift of 3.56 for Ly-α and 3.53 for CIV. The very strong absorption in the blue wing of the feature identified as Ly-α has probably shifted the measured wavelength to the red of the true peak. For this reason we do not regard the slight difference in the measured redshifts of the lines as being significant. We found no other acceptable fit to the observed ratio of wavelengths that could not be rejected because of the absence of other strong lines in observed spectral regions. The presence of many strong absorption lines would also suggest a large redshift, say $z \gtrsim 2$. We therefore conclude that the emission line redshift is near 3.53. The position of several features assuming a redshift of 3.53 have been marked on Fig. 1.

It is common for large redshift QSOs to have absorption features; OQ172 seems to have one of the richest absorption spectra known. The heavy absorption shortward of Ly-α increases the difficulty of identifying emission features in that spectral region. There is some indication of emission at the expected position of Ly-β and OVI but spectra with higher resolution will be needed to reduce the line blending and establish the continuum level before any such identification can be regarded as secure. In any case, there is no doubt that the continuum radiation remains strong far to the violet of the position of the Lyman edge in the emission line redshift system, a surprising result for an object with such strong absorption features. The strongest lines near the Ly-α emission peak have the correct separation to be identified with the CIV λ1549 doublet at $z=2.56$, raising the possibility that the absorption line systems have a much lower redshift than the emission line system. The Lyman continuum absorption associated with these features would then begin at the extreme violet end of our data where the signal-to-noise ratio is poor. It is also possible, in a gas with little turbulent motion, to have a substantially higher optical depth in Ly-α than in the Lyman continuum. One could therefore have strong, narrow, Ly-α absorption features without having appreciable absorption in the Lyman continuum. A programme to obtain higher resolution spectra of this object is now under way and should help resolve some of these questions.

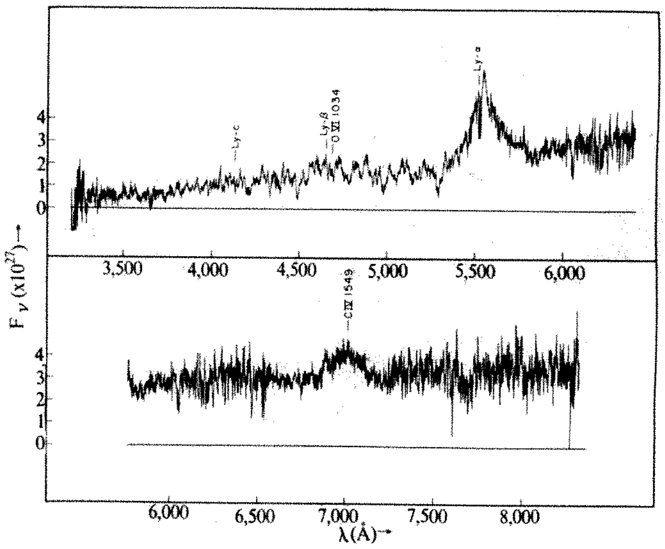

Fig. 1 Image tube scanner spectrum of OQ172. Units of F_ν are erg cm^{-2} s^{-1} Hz^{-1}.

OQ172 is very bright. It was estimated by Véron[4] to be magnitude 17.5. Our calibrated spectra, which should be accurate to ±20%, indicate that the V magnitude of the continuum is about 17.9. The strong continuum level, extending well into the ultraviolet, is in agreement with the blue classification given to OQ172 by Véron[4]. Although it is bright at short radio wavelengths it is too faint to appear in the 4C catalogue, otherwise it satisfies all the normal criteria for classification as a QSS. Other, similar objects presumably exist and could be discovered by the proper survey techniques without requiring highly accurate radio positions.

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Intensity and Extent of Rain on Earth-Space Paths

Existing broadband satellite communications systems operate at carrier frequencies such as 4 and 6 GHz where bandwidths of about 0.5 GHz are available. At frequencies of 15 to 40 GHz, however, bandwidths of the order 3 GHz are possible but propagation through rain presents difficulties because there is significant absorption and scattering at the higher frequencies[1].

Here we examine attenuation data obtained simultaneously at 16 and 30 GHz using a Sun tracker[2] in New Jersey. The Sun is a source of quite constant power at these frequencies. The instrument is capable of measuring rain attenuation up to 35 dB at both frequencies with a time constant of 2 s. Operation is of course limited to the daytime, but the data base thus obtained amounts to about 4,000 h yr^{-1}.

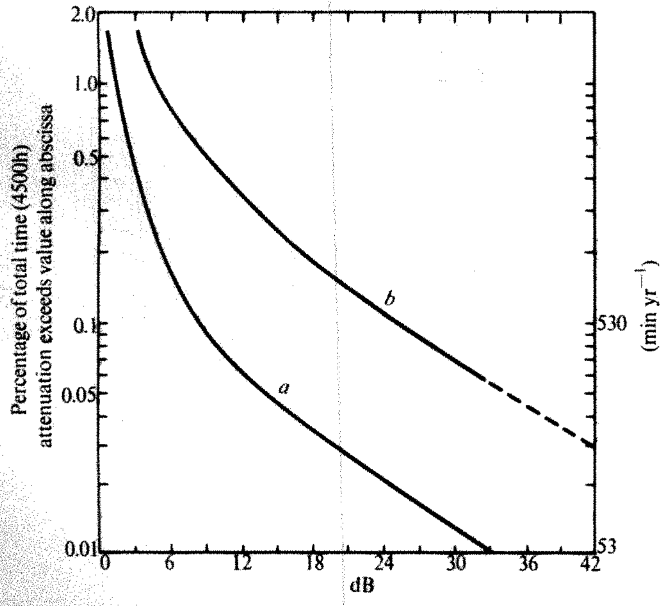

Fig. 1 Distribution of attenuation by rain measured simultaneously (in daytime) at 16 (*a*) and 30 (*b*) GHz by the Crawford Hill Sun tracker during 1968.

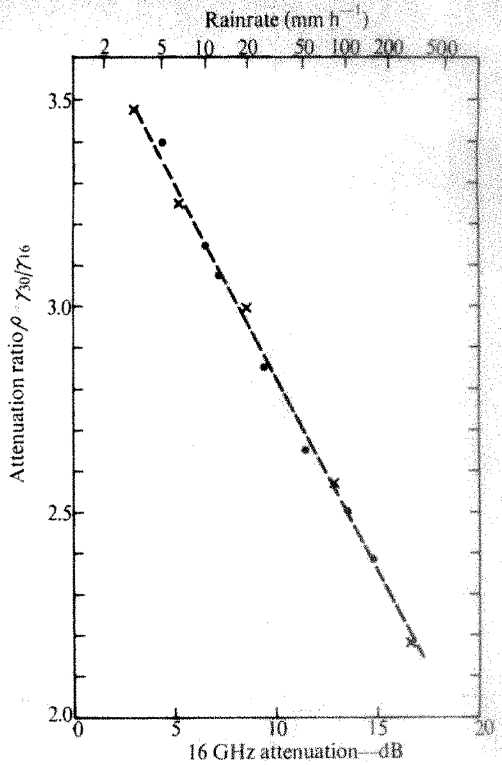

Fig. 2 Measured ratio of attenuations, obtained from the distributions of Fig. 1 against attenuation, and calculated ratio of attenuations against rain rate. ×, ρ_m, measured; ●, ρ_c, calculated.

Comparison of the statistical behaviour of signals at two or more frequencies propagated over a given path has been useful in the past for determining the transmission properties of the troposphere at both radio[2–5] and optical[6] frequencies. In the present case, the ratio of attenuation at 30 GHz to that at 16 GHz, obtained from cumulative distributions of attenuation, is used to estimate the intensity and extent of rain showers on an Earth-space path. To do this, it is necessary to specify a size distribution for the raindrops. We choose the classical (measured) Laws-Parsons distribution[7] which represents adequately the drop sizes in most rains. A salient property of this size distribution is that the more intense the rain, the more abundant are drops of large diameter.

The cumulative probability distributions of attenuation at 16 and 30 GHz for the days of 1968 are shown in Fig. 1. Because the radiation from the Sun at both frequencies is received on the same antenna (beamwidths 0.9 and 0.5°), the attenuated signals resulting from rain showers passing through the Earth-space path are time-wise in step; attenuation maxima and minima coincide. Therefore the ratio, ρ_m, of measured attenuations at a given probability is significant; for example, at the 0.1% level, $\rho_m = \gamma_{30}/\gamma_{16} = 25.5/8.5 = 3.0$. When this procedure is carried out for a number of probability levels, one obtains the crosses plotted in Fig. 2 which show that the measured ratio, ρ_m, decreases from 3.5 to 2.2 as the 16 GHz attenuation increases from 3 to 16 dB.

We now assume that the rain rate is constant over a segment of the path and that the size distribution of the raindrops is, at least on the average, the Laws-Parsons distribution. The calculated ratio, ρ_c, for various rain rates can then be obtained from computations based on the Mie theory[7]. In Fig. 2 ρ_c is plotted against rain rate as solid dots; the calculated attenuation ratio decreases from 3.5 to 2.4 as the rain rate increases from about 5 to 150 mm h^{-1}. This behaviour follows from the greater abundance of large drops at high rain rates.

Clearly, both plots are well represented by the dashed line in Fig. 2; thus a direct comparison can be made between them in the following sense. When the 16 GHz attenuation is 8 dB, the measured attenuation ratio is 3.0. But that ratio is represented by a rain rate of 15 mm h^{-1}; therefore, when the attenuation on the path is 8 dB, the rain rate on the path is, on the average, 15 mm h^{-1}. For an attenuation of 13.5 dB, $\rho = 2.5$, the apparent rain rate is 100 mm h^{-1}, and so on.

The equivalence between attenuation and rain can be used to derive a cumulative distribution of apparent rain rate; thus from Figs 1 and 2, one obtains Fig. 3. The abscissa is labelled "apparent" rain rate, R_a, for two reasons: to ensure that it is not confused with path-average rain rate, and because, on an Earth-space path, it represents density of rain rather than an actual rate, the velocity of fall in many convective rains being unknown.

If this derived distribution is compared with the point rain rate as measured by a rain gauge located at some point near the antenna, it is found that the probability occurrence of the high rates is much greater on the path than at the point. This is not surprising because it is obvious that a rain shower, appearing randomly over an area, is much more likely to intersect a line than to encompass a point.

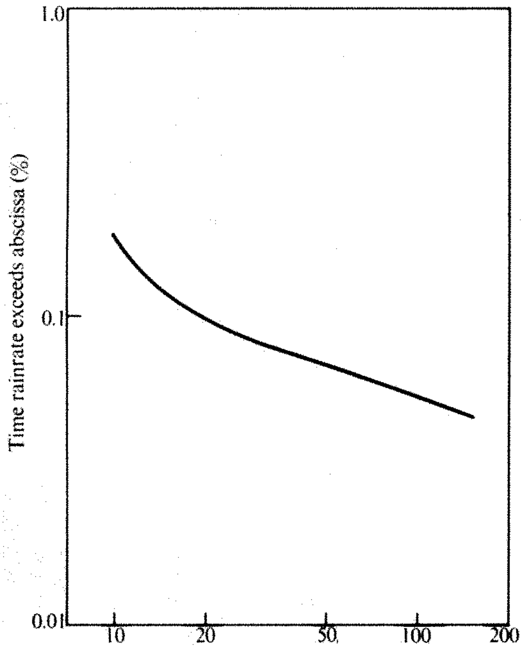

Fig. 3 Cumulative distribution for the apparent rain rate on the Earth-space path.

Up to this point, we have only been concerned with the ratio of attenuations. But the absolute value of attenuation is also known, as is the relationship between attenuation and path length, for a given uniform rain rate. If l is the extent of a rain of rate R_a on a path of arbitrary overall length, the attenuation is simply

$$\gamma_p = l\alpha(R_a)$$

where $\alpha(R_a)$ is the known attenuation coefficient[7]. γ_p is known from the measurements (Fig. 1) and $\alpha(R_a)$, for the same probability level, can be calculated using Fig. 3. Therefore the extent or path length, l, through the rain of apparent rate R_a can be obtained using the above relationship; it is shown in Fig. 4.

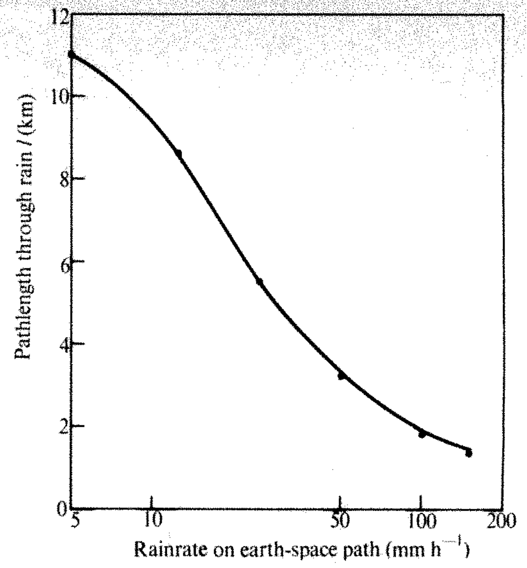

Fig. 4 Extent of rain on the Earth-space path against rain rate.

Figure 4 shows that at low rain rates the extent of the "cell" on the path is large, 10 km or more. On the other hand, when rain rate exceeds 100 mm h^{-1}, the extent of the cell is less than 2 km. Thus we end up with the common-sense result that the less intense rains on the average cover large areas whereas heavy showers are quite limited spatially. One can also plot the extent of the rain as a function of attenuation at 16 or 30 GHz and the behaviour is similar to that of Fig. 4—high attenuations are caused by relatively small cells. (This fact is basic to the technique of path diversity in millimetre-wave satellite communication systems.)

What is the practical significance of the method outlined above? A point on the plot in Fig. 4 gives the path length through a shower of given rain rate, and each point is associated with a certain probability. Therefore one can use those data to compute the attenuation statistics at any desired frequency where the Laws-Parsons drop-size distribution is applicable, resulting in a cumulative attenuation distribution for that frequency. Evans[8] has applied the method in satellite-system calculations.

There are at least two criticisms to be made of this technique. First, the basic data (Fig. 1) were taken using a Sun tracker which encounters paths of various overall length through the atmosphere during a day. Dual-frequency data taken on a path at a fixed elevation angle, such as to a synchronous satellite, would be more satisfactory for the analysis. Second, the Laws-Parsons drop-size distribution has been assumed throughout. There is evidence[5], however, that that distribution is satisfactory on terrestrial paths at the frequencies of interest. On the other hand, comparison of the plots in Fig. 2 promotes some confidence in its applicability in the present case. It is not known how sensitive the method is to a given drop-size distribution.

The technique amounts to remote probing, in the statistical sense, of the gross features of the rain medium; it is applicable to terrestrial as well as Earth-space paths.

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Stress Diffusion from Plate Boundaries

ACCORDING to global tectonics, the outer relatively strong layer of the solid Earth (lithosphere) is divided into seven major and several minor discrete plates separated from each other by the Earth's mobile belts. The mechanism of global tectonics is believed to depend partly or wholly on the application and release of strain energy at mobile belts which lie along plate margins. The isostatic response of the lithosphere to application or removal of a wide surface load such as an icecap is delayed by the viscous response of the underlying asthenosphere to elastic bending of the lithosphere[1]. By analogy, the viscous drag of the asthenosphere should also delay the response of the lithospheric plates to relief or application of horizontal boundary stresses on a plate edge, as indicated by an earlier calculation made by Elsasser[2]. We have investigated this using a simple plane two-dimensional model of an elastic plate underlain by a viscous layer. We confirm that the viscous shearing stress of the asthenosphere on the base of the lithosphere is a significant factor in causing stress to diffuse through the lithosphere. We also predict the possible occurrence of a new type of strain wave in the lithosphere, with some resemblance to Voigt waves[3,4].

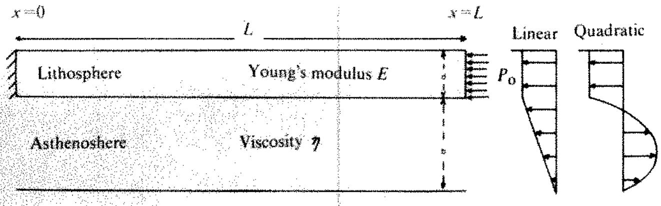

Fig. 1 Two-dimensional model of the lithosphere overlying the asthenosphere, with linear and quadratic velocity-depth distributions shown at the right side.

Our theoretical model consists of a horizontal two-dimensional elastic plate of length L, thickness d and Young's modulus E representing a lithospheric plate, underlain by a viscous layer of thickness b and Newtonian viscosity η representing the asthenosphere (Fig. 1). The viscous layer overlies a rigid and stationary half-space. The Earth's curvature is neglected and plane stress is assumed. We study the variation of horizontal pressure P and horizontal displacement $u(x,t)$ in the direction of the x axis as a function of distance x and time t. Inertial forces were included in some of our calculations but their effect was negligible for parameters compatible with the Earth.

By considering the horizontal forces acting on a small length of the elastic plate and neglecting inertial forces, one obtains an equation of motion for the displacement $u(x,t)$ of the part of the plate at point x at time t. This is[2]

$$\frac{\partial^2 u}{\partial x^2} = \sigma \frac{\partial u}{\partial t} \qquad (1)$$

where $\sigma = \eta/bdE$ for an assumed linear velocity-depth distribution across the viscous layer or $\sigma = 2\eta(3d+2b)/b^2dE$ for a quadratic distribution such that no net transport occurs across a section perpendicular to the x axis. The horizontal variation of $P(x,t)$ within the elastic plate implies the existence of a bending moment which will cause small vertical movements, but we ignore this effect in the present exploratory investigation.

Equation (1) also applies to the propagation of a horizontal shear stress applied at the edge of the plate provided that E is replaced by the rigidity modulus μ. Equation (1) has identically the same form as the unidimensional equations of heat conduction or diffusion. The equation can be solved for given initial and boundary conditions by standard methods[5].

A simple investigation which gives much insight is to determine the response of an elastic plate of semi-infinite length to a sinusoidal boundary pressure $P = P_0 \sin wt$ of period $T = 2\pi/w$ applied at the end $x = 0$. The appropriate solution of equation (1) is

$$u(x,t) = -\frac{P_0}{\sqrt{2}kE} e^{-kx} \cos\left(wt - kx + \frac{\pi}{4}\right) \qquad (2a)$$

and

$$P(x,t) = P_0 e^{-kx} \sin(wt - kx) \qquad (2b)$$

where $k = \sqrt{\tfrac{1}{2}w\sigma}$. This represents a train of stress (or strain) waves of period T whose maxima and minima travel along the x-direction with velocity $v = w/k$, with amplitude falling off exponentially with distance. Defining the penetration X as the distance to the point where the amplitude falls off to e^{-1} of its value at $x = 0$, we have for the linear velocity in the viscous layer

$$X = \sqrt{\frac{ETbd}{\pi\eta}} \text{ and } v = 2\sqrt{\frac{\pi Ebd}{\eta T}} \qquad (3)$$

We calculated representative values to indicate the order of magnitude of the penetration and velocity assuming $d = 80$ km, $b = 250$ km, $\eta = 2 \times 10^{21}$ P, and $E = 10^{12}$ dyne cm^{-2} (Table 1).

Table 1 Representative Values of Model Parameters

T (yr)	X (km)	v (km yr^{-1})
1	10	62.8
10^2	100	6.28
10^4	1,000	0.628
10^6	10,000	0.0628

For the quadratic velocity-depth distribution in the viscous layer, the values of X and v in the table should be divided by 2.43.

One implication of this result is that a sudden change of the boundary stress at the edge of a plate (such as might result from an earthquake) would not be expected to penetrate into the interior of the plate except over a long period of time, except insofar as the asthenosphere can respond elastically[6].

Another implication is that one might expect to observe stress waves in the vicinity of plate boundaries where boundary stresses vary with time, travelling away from the boundary with a velocity inversely proportional to the square root of period. The penetration of waves of period of up to 100 yr would be restricted to a few hundred kilometres from the plate margin. An example of such stress waves has been presented by Kasahara[7], based on tiltmeter records at two stations in Japan about 20 km apart. Crustal strains of dominant period about one to four years appear to migrate from east to west (away from the surface position of the Benioff zone) at a velocity of about 20 km yr^{-1}. The velocity is in good agreement with the predictions of the table above, although the origin of the stress variation appears to be closer than the Japan trench which is 200 km away.

If our model is correct, search for secular strain variations should be made in the vicinity of plate boundaries but not in the interior regions of plates.

We now investigate the response of the elastic plate of finite length L to the application of a constant pressure at one end representing an ocean ridge, and to the release and subsequent rebuildup of strain at the other end, representing a compression plate boundary such as an island arc-trench system. Although the models must be treated as severe oversimplifications, particularly for the release of strain, they do give significant insight into the relevant processes.

For the first problem, the end of the plate at $x = 0$ is regarded as fixed and represents a compression boundary. A constant

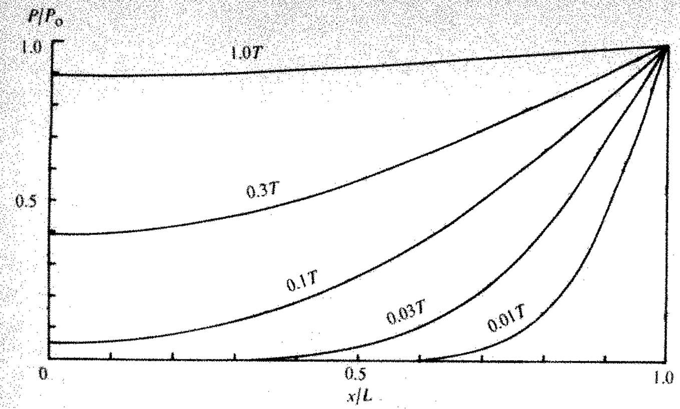

Fig. 2 The horizontal pressure distribution in the lithosphere plotted for a series of values of time after application of a pressure P_0 at the end $x=L$ at time $t=0$, where time $T=\sigma L^2$. If $L=10{,}000$ km, $d=80$ km, $b=250$ km, $\eta=2\times10^{21}$ P and $E=10^{12}$ dyne cm^{-2}, then $T=316{,}900$ yr for the linear velocity-depth distribution and $T=1.87\times10^6$ yr for the quadratic distribution.

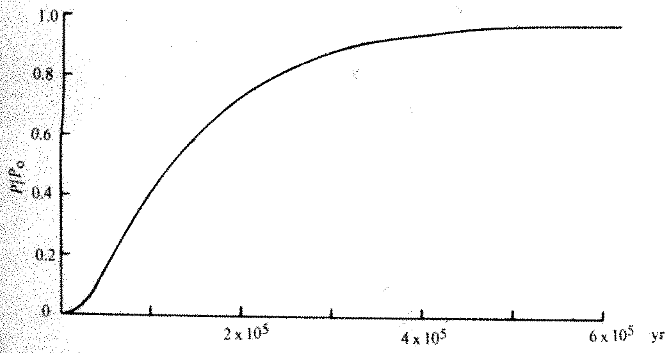

Fig. 3 Graph showing the buildup of horizontal pressure P at $x=0$ as a function of time after application of pressure P_0 at $x=L$ for the linear velocity-depth distribution assuming the values of parameters given in legend of Fig. 2.

pressure P_0 is instantaneously applied at the end $x=L$ representing the ocean ridge at time $t=0$. Equation (1) must then be solved for the initial condition $u=0$ at $t=0$ $(0\leqslant x<L)$, and for the boundary conditions $u=0$ at $x=0$ $(t>0)$ and

$$\left(\frac{\partial u}{\partial x}\right)_{x=L} = -P_0/E,\ t>0.$$

The resulting pressure distribution along the plate has been plotted in a non-dimensional form in Fig. 2 for values of time t shown on each wave, where $T=\sigma L^2$. Figure 3 shows the buildup of pressure at the fixed end A as a function of time for specific values of the dimensions and physical properties (it can be scaled readily for other values). These examples show that it takes about 10^5 to 10^6 yr for the pressure to approach the value P_0 throughout the plate if $L=10{,}000$ km, and of the order of 10^3 to 10^4 yr if $L=1{,}000$ km.

If the end A is interpreted as a compression boundary of a plate, then as pressure increases at A (Fig. 3) it will eventually reach a critical value P_c at time τ when resistance is overcome and the edge of the plate is displaced (for example down a Benioff zone). In our final model, we examine how the pressure will rebuild at A after such stress relaxation. We simulate stress relaxation at and near A by assuming an instantaneous displacement $u_+ = -\dfrac{P_c s}{E}\,e^{-x/s}$ at time τ, where s is a parameter which controls the fall-off of the stress changes with distance from the origin. A computed plot of the buildup of pressure at A after stress relaxation, assuming $P_c=526$ bar and $s=3$ km, is shown in Fig. 4. This shows that the stress in the vicinity of the boundary builds up again in a period of 1 to 10^3 yr. When the pressure again reaches the critical value the relaxation will be repeated, and because the timescale in Fig. 3 is so much larger than in Fig. 4 one would expect the initial conditions after the second relaxation to be almost identical to those after the first. Thus the pressure at A would be expected to cycle as in Fig. 5.

In the example (Fig. 4), the instantaneous displacement at $x=0$ for $s=3$ km is 150 cm and the release of strain energy is 0.6×10^{18} erg cm^{-1} length perpendicular to the model. Our model of stress release is a serious oversimplification, and in practice the stress drop would only be partial and would vary with depth. One would expect a smaller instantaneous displacement and release of strain energy occurring more frequently. In spite of our simplification of the problem, it is easy to see that plates can move forward at velocities of the order of 6 cm yr^{-1} by this mechanism.

On our model the plates are driven by horizontal pressure applied at or near ocean ridges. We have shown that short period cyclical variations of pressure at the ridges do not penetrate into the interior of a plate and that persistent changes in pressure take of the order of a million years to penetrate across a large plate. At the compression boundary, instantaneous release of strain energy probably only affects a few tens of kilometres at most from the boundary and the stresses then build up towards their original value over a period of the order of 1 to 100 yr. Thus the model predicts that release of strain energy at a compression boundary will occur periodically and this part of the plate will move forward in a series of jerks. The principal interior part of the plate acts as a reservoir for strain energy, isolating the opposite boundaries from each other, and moving forward at a uniform velocity as long as the applied pressure at the ridge averaged over a suitably long period remains constant.

Another possibility is that lithospheric plates are driven by

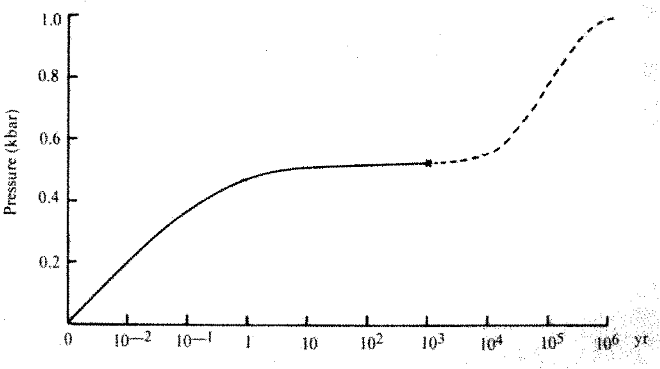

Fig. 4 The increase in horizontal pressure P at $x=0$ after strain release as described in text. Failure would be expected to occur again at X.

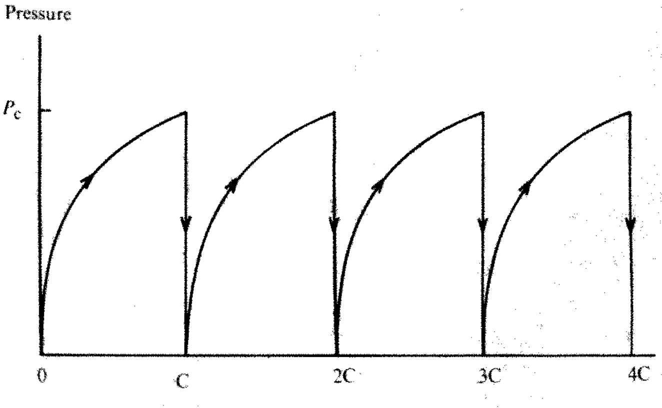

Fig. 5 Diagram to illustrate qualitatively the periodic release of strain at a compression boundary at time interval C, as predicted by our model.

drag of underlying convection currents. We have not studied this model of plate mechanism, but similar considerations would be expected to apply to the response of the plate to changes in the driving stresses and to release of strain energy at compression boundaries.

We have shown that stress suddenly applied or released at a plate boundary cannot instantaneously affect the whole plate but diffuses through it over a period of the order of a thousand to a million years depending on dimension. The crucial factor causing this slow stress migration is the viscosity of the asthenosphere. If the stress variation at a plate boundary is periodic, then a decaying train of stress waves will pass into the plate with velocity depending on period and physical properties of the order of 0.1 to 10 km yr^{-1}. Finally, if our model of stress diffusion in the lithosphere is substantiated by future observations, it may become, in association with the theory of creep wave propagation along transform faults[8], an important factor in prediction of earthquakes and volcanic activity near compression boundaries.

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Evidence for Buffering of Dissolved Silicon in Fresh Waters

THE widely used colorimetric method for determining dissolved silicon in natural waters measures only monomeric or low molecular weight polymers of silicic acid[1]. But there seems little reason to believe that appreciable amounts of higher, and therefore unreactive, polymers exist in the sea or most terrestrial waters[2,3].

Livingstone[4], in the most extensive compilation of river water analyses to date, obtains a worldwide mean dissolved silicon concentration of 13.1 mg SiO_2 l^{-1}. These data show that the concentration of silicon in rivers is remarkably constant compared with other major dissolved constituents (for example, the coefficient of variation for all tabulated silicon values is approximately one fifth that for total dissolved salts). This finding is reinforced by the discovery[5] that 90% of groundwater supplies in the USA have silicon concentrations in the range 7 to 43 mg SiO_2 l^{-1}, whereas sulphate shows a variability eight times greater.

Many major constituents in river water show an inverse relationship between concentration and discharge[2]. This is normally thought to be caused by dilution of baseflow by storm runoff. In the case of silicon the concentration in solution is often insensitive to change in discharge compared with other major components. For example, of seven US rivers cited by Davis[5,6], six show no discernible change in dissolved silicon with discharge, and only one (Anchor River, Alaska) exhibits a dilution with increased river flow. A similar lack of correlation was found in studies of five small catchments in the Swiss Pre-alps[7]. Gibbs[8] showed that in the Amazon silicon levels of about 9 to 12 mg SiO_2 l^{-1} were maintained in spite of considerable seasonal change in discharge. For a small catchment in Maryland, dissolved silicon decreased by 10% (9.4 to 8.4 mg SiO_2 l^{-1}), through a storm event[9], although the stream discharge increased by 284%. Detailed sampling in the Hubbard Brook experimental catchments[10] showed that, although levels of dissolved silicon were inversely related to discharge, the slope of the regression line was considerably less than that expected from dilution of baseflow by rainwater containing a negligible amount of silicon. A study of rivers in Norfolk, England[11], shows that, except in periods of high plant productivity, dissolved silicon varied within very narrow limits (10 to 12 mg SiO_2 l^{-1}) although the discharge showed much larger variations.

These results indicate that relative to other major dissolved constituents, river water silicon tends to be remarkably uniform on a worldwide basis and shows little response to change in discharge. They suggest that some controlling mechanism exists for silicon in natural waters which does not apply to other major components. Diatoms and other plankton can vastly deplete dissolved silicon, for example to less than 1 mg SiO_2 l^{-1} during the spring bloom in some Norfolk rivers[11]. Silicon is also stored in some catchments by terrestrial macrophytes. But neither of these biological mechanisms seems adequate to maintain near constant concentration through the rapidly changing discharge of a storm hydrograph or to explain the small global inter-river variability.

An abiological buffering mechanism seems more likely. Dissolution of silicate minerals, although the ultimate source of most silicon in solution, is unlikely to be sufficiently rapid in terms of individual hydrographic events. Davis[6] suggests that desorption of silicic acid from soil particles is more likely. In laboratory experiments[12] hydrated oxides of aluminium, iron, manganese and magnesium can adsorb silicon from dilute solutions (3 mg SiO_2 l^{-1}). The cycle of silicon in some lakes seems to be intimately linked to the formation and dissolution of iron and manganese oxide solid phases, in response to changes in redox potential[13,14]. Reactions of this type may explain the large scale removal of dissolved silicon, above that accounted for by biological processes, found in Lake Biwa, Japan[15].

The behaviour of dissolved phosphorus seems to be similar in many ways to that of silicon. Deoxygenation of lake sediments results in the release of both elements[13], and in the Amazon basin rapid equilibrium is established between sediments and dissolved phosphorus in the river water[16].

For soils, there is laboratory evidence that levels of dissolved silicon are controlled by sorption reactions involving oxides of iron and aluminium[17,18]. These studies, which have used both natural soils and pure solid phases, show that both adsorption and desorption are possible. Beckwith and Reeve[18] suggest that for soil waters much rapidly soluble silicon is derived from sorption sites in the soil and not from solution of specific silicon compounds.

Although dissolution of solid phases and biological activity are important, they cannot fully explain the low temporal and spatial variability found for dissolved silicon levels in fresh waters. It seems that sorption reactions involving dissolved silicon and solid phases are a more likely buffer mechanism controlling the concentration of dissolved silicon in rivers, lakes and soils.

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Grass Roots at the Base of the Neogene

THE history of life on Earth seems to have been punctuated by fairly swift changes in ecosystems, brought about initially by tectonic or cataclysmic events. The flora, being at the base of the food chain, would have a secondary effect on faunal evolution when undergoing even slight modifications. Such an instance may be found at the end of the Mesozoic, when the oncoming angiosperms must have greatly modified faunal ecosystems on the land. But it would be incorrect to assume that these changes had no relevance to seafloor communities, especially with regard to the appearance of grasses.

The Graminae, although appearing in the Late Cretaceous, did not become abundant until early Neogene[1]. This group must have played a fundamental role in the shaping of Cainozoic ecosystems by providing a vast ground level food supply capable of being continuously cropped by herds of mammals, whilst also providing niches for many smaller organisms. It must also have modified the geomorphology by stabilization of the soil surface. The Marram grass *Ammophila* is a present day example, where stabilization of coastal dunes has enabled the establishment of complex ecosystems on formerly barren sands. Similar effects follow the appearance of marine grasses such as *Thalassia* in backreef communities in the West Indies[2]. It follows that remarkable changes must have occurred after the initial colonization of the seafloor by the Graminae. Backreef and inshore sediments would have been stabilized, accompanied by an accumulation of silt and mud-sized particles, an increase in the organic content of sediments and thus of deposit feeding organisms. Marine grasses would also have provided new sources of food for the macrofauna and microfauna and new means of dispersal for benthonic and phytal organisms, the latter perhaps resulting in a decreasing chance of isolation and speciation.

Unfortunately, the date of the appearance of marine grasses is not traceable by palynology because they produce pollen without exine, and so are not fossilized[1]. Their former presence can nevertheless be deduced by sedimentology and palaeontology. Sediments from these meadows are characterized by a bimodal distribution of grain size, having abundant trapped silt together with much coarse biogenic material, especially gastropods, serpulids, ostracods and foraminifera. My analysis of foraminifera living on seagrass from the Caribbean[3] has shown that they consist largely of different species to those dwelling on the sediment and are therefore useful as palaeoecological indicators. Examination of the published fossil record suggests that this seagrass association occurred for the first time in the early Miocene Alum Bluff stage of Florida, the microfauna of which has been described by Puri[4,5]. It was also at this time that the typically phytal foraminifera *Sorites*, *Amphisorus* and *Marginopora* first appeared, becoming widespread throughout the tropics. On the debit side, the Miocene witnessed the extinction of the larger orbitoid and nummulitid foraminifera. This event may also have been precipitated by the appearance of seagrass, for they were probably adapted to the less stable sediments of Palaeogene reefal areas.

It therefore seems credible that the changes from Palaeogene to Neogene benthonic biofacies can be partly attributed to the effects that encroaching marine grasses must have had on the ecology.

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BIOLOGICAL SCIENCES

Synthesis of a Matrix-supported Enzyme in Non-aqueous Conditions

IN recent years the chemical and physical properties of matrix-supported enzymes have received considerable attention in the chemical literature[1,2]. The discovery that biologically active compounds can be fixed artificially to insoluble polymeric supports has generated an interest in these materials which is manifest in their wide applicability to the study of complex biological systems. This is so because, in general, the immobilization of active proteins has been shown to produce several significant chemical and physical changes in the protein relative to its native-state counterpart. For example, certain enzymes, which in an isolated extracellular environment exhibit relatively low thermal stability, can be materially stabilized by attachment to an inert polymeric support. Furthermore, chemical properties, which are manifest in the mechanism of action, can be altered significantly by the technique, for example reaction kinetics and substrate specificity.

Applied research ranges from the study of matricized enzymes as *in vitro* models for complex *in vivo* systems to, for example, their more practical use in continuous assay systems for glucose, urea and anticholinesterase compounds[3]. The uses of matrix-supported enzymes in the continuous resolution of racemic mixtures and continuous isomerizations and degradations of saccharides have become important industrial processes[4].

In general, four basic procedures are used for preparing immobilized enzymes: (1) adsorption on synthetic resins; (2) entrapment in gel lattices; (3) covalent binding through nucleophilic groups of the protein with electrophilic functional groups on inert polymers, and (4) covalent cross-linking with certain bifunctional reagents. Moreover, most recent research dealing with the synthesis of insolubilized enzymes has emphasized procedures which effect protein attachment to the support through covalent bonds. Typically, such synthetic routes as azide displacement, diazo coupling, and binding through cyclic imidocarbonates are used[2,5,6]. Invariably though, the choice of activation procedure for a given polymeric support has been made so as to be consistent with covalent bond formation in aqueous media only. Obviously, this condition places a severe restriction on the design of chemical reactions by which the protein is covalently bound to the support. As a result, the many chemical techniques available to the synthetic chemist have not been utilized in the preparation of matrix-supported enzymes. One purpose of the study reported here was to determine if this "aqueous restraint" was justified or only assumed as part of an historical artefact, since biologically active materials are typically studied in aqueous (buffered) media, ostensibly to mimic their natural environment*.

Our approach to the first non-aqueous synthesis of a matrix-supported enzyme is outlined in Fig. 1. Because enzymes (for example, lysozyme) typically have a low solubility in organic solvents it was convenient to perform the coupling reaction using a support which could first be dissolved in an appropriate organic solvent, then activated to yield a soluble matrix containing the reactive species, thus binding the insoluble proteins in a two phase reaction system. For this reason, the linear 4-vinylbenzoic acid-styrene copolymer[10] (I) was chosen as the support. (All attempts to prepare a homopolymer of 4-vinylbenzoic acid[11] yielded only very low molecular weight polymers, which did not possess solubility characteristics consistent with the method outlined in Fig. 1.) The copolymer prepared in a 1:3 molar ratio from the monomers displayed solubility characteristics consistent with final isolation of the polymer-enzyme complex by a non-solvent precipitation process. The scheme outlined in Fig. 1 allowed complete removal of all unreacted enzyme before product precipitation and purification, thus avoiding enzyme adsorption to the precipitated polymer-enzyme complex. The model enzyme—lysozyme—was chosen primarily because of its ready availability in highly purified form. N,N′-carbonyldiimidazole was chosen as the coupling reagent for two reasons. First, it has been shown to be very effective in peptide synthesis[12,13], reacting rapidly and essentially quantitatively with carboxylic acids at room temperature to yield intermediate amides (Fig. 1). Coupling with amino (or alcoholic groups)[12,13] can then proceed by displacement of imidazole. Second, the by-products of the coupling procedure are innocuous.

The experimental procedure was as follows: a copolymer of styrene (4.22 g; 0.0405 mol) and 4-vinylbenzoic acid[14] (2.00 g; 0.0135 mol) was prepared by treating a mixture of the monomers with 50 mg of benzoyl peroxide at 80° C. After 24 h a clear, colourless, hard polymer formed which was removed from the polymerization vessel, ground to a fine powder, and used without further purification. For the enzyme binding reaction, 200 mg of copolymer was dissolved in 5 ml of freshly distilled N,N-dimethylformamide. N,N′-carbonyldiimidazole[12,13] (70 mg; equivalent to the amount of *p*-carboxystyrene contained in the copolymer assuming a statistical distribution of the monomer in the copolymer) was added and the solution was stirred for 30 min at room temperature. Crystalline egg-white lysozyme (20 mg) was added in one portion and the mixture was stirred for 24 h at room temperature. At the end of the stirring period, unreacted lysozyme was still visible in the mixture. The latter was removed by centrifugation and the organic supernatant was separated. At this point in the reaction sequence, it was frequently noted that slight cooling of the dimethylformamide reaction mixture (15° C) resulted in precipitation of the polymer-enzyme complex. This occurred in cases where the concentration of the product was more than about 40 mg ml^{-1} in dimethylformamide solution. Similar reactions carried out on polymer solutions containing enzyme, however, without added N,N′-carbonyldiimidazole, failed to form precipitates even at 5° C. Polymer solutions at 40 mg ml^{-1} concentration treated with N,N′-carbonyldiimidazole, but not enzyme, likewise gave no precipitation when cooled. These observations are consistent with the expected large increase in molecular weight as the coupling reaction proceeds. The copolymer-enzyme complexes were finally isolated by dilution of the dimethylformamide solution into water or buffer (*p*H 6.2 phosphate, 0.2 M). White granular precipitates were thus obtained, subsequently removed by filtration, and washed thoroughly with buffer to remove the last traces of organic solvent.

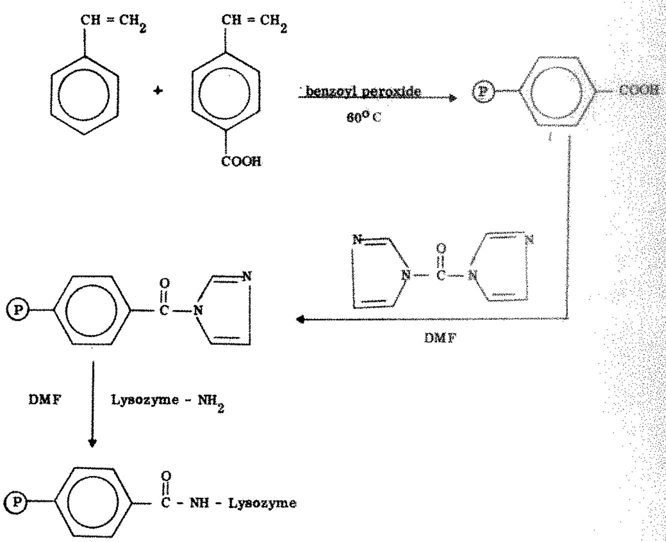

Fig. 1 Non-aqueous synthesis of matrix-supported lysozyme. DMF, Dimethylformamide.

The amount of protein bound to the polymer was estimated by the method of Bramhall *et al.*[15]. Typically, 5–7 mg of lysozyme could be bound to 200 mg of copolymer. The activity of the bound protein toward *Micrococcus lysodeikticus* was determined by the method of Shugar[16] and is expressed in spectrophotometric units (SU) as follows:

$$SU = \frac{\Delta A/\text{min} \times 1{,}000}{\text{mg protein}}$$

Native lysozyme exhibited an activity of 10,500 SU, whereas the matrix-supported protein gave an activity of 4,330 SU. Thus, on a weight basis, the matrix-supported material retains about 40% of native activity.

The fact that the enzyme "survived" the application of a rigorous chemical synthesis in anhydrous conditions is significant for numerous reasons. In the first instance, the manipulation of enzymes in mixtures of water and organic solvents is known to result in an "unfolding" of the enzyme in a way which exposes the hydrophobic portion of the molecule[17]. The latter effect may be minimal in the present case since the protein was not in solution, but by judicious choice of organic solvent parameters, the degree of unfolding might well be controlled, thus allowing subsequent binding while the protein assumes various predetermined and variable conformations. In those conditions it should be possible to design appropriate chemical syntheses to allow a highly selective chemical binding process to occur, in fact, one involving hydrophobic groups preferentially in the presence of hydrophilic groups. We believe that these properties can be exploited to both theoretical and practical advantage.

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* The literature contains a few cases where proteins have been subjected to chemical treatment in anhydrous conditions. For example, several biologically active proteins have been shown to be both soluble in and recoverable from anhydrous hydrogen fluoride[7] or anhydrous trifluoroacetic acid[8] without loss of enzymatic activity. Also, Siegel and Roberts[9] have briefly reported the activities of the redox enzymes peroxidase and catalase in a number of anhydrous organic solvents. The feasibility of carrying out chemical reactions for the immobilization of proteins in strictly anhydrous media has, however, not been examined to our knowledge.

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Rate Constants of Electron T Processes in Solution: Depen on the Redox Potential of the Acceptor

ONE-ELECTRON oxidation–reduction reactions h lished in several enzymatic reactions, and ele has been found to be necessary in phosphoryla

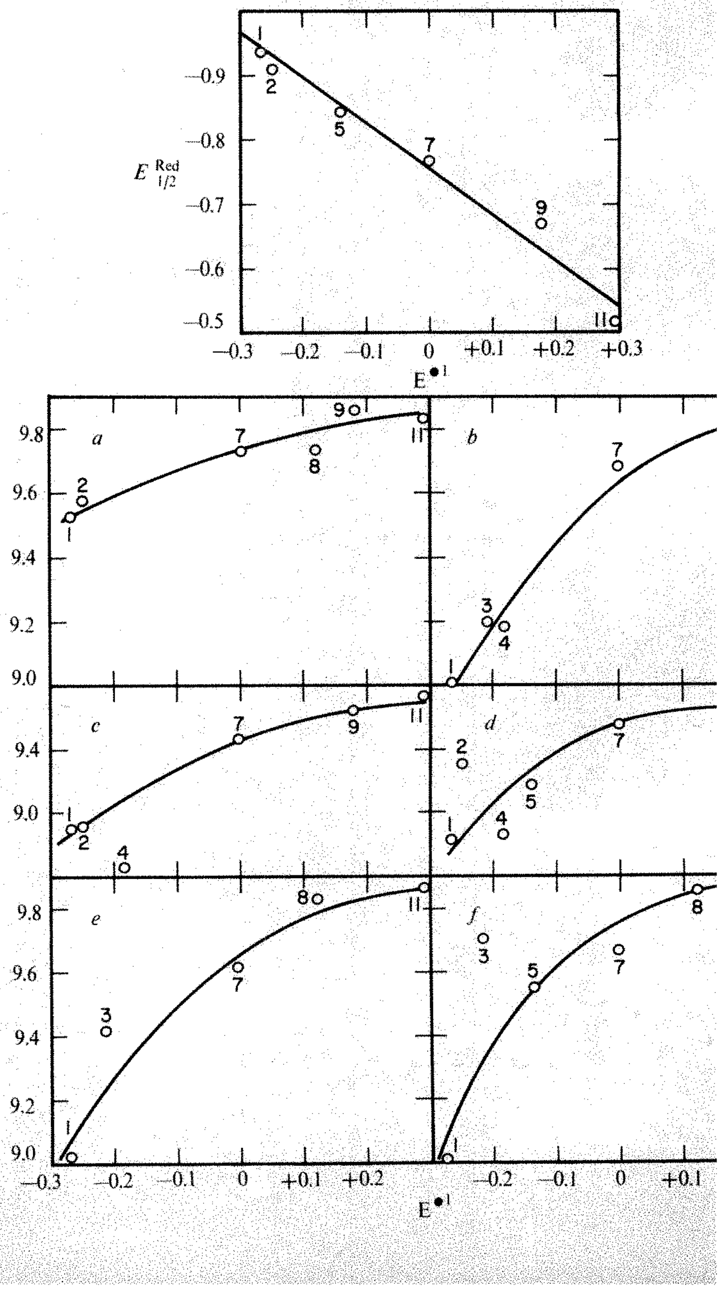

Fig. 1 Dependence of log *k*, the electron transfer rate constants of various free radicals (produced by reaction of substrate with e^-_{aq}) upon the $E^{\bullet 1}$ values of the acceptors. The numbers refer to the acceptor used. Included is the correlation between $E^{\bullet 1}$ and the half-wave reduction potential of some of the acceptors (taken from ref. 17). *a*, Nicotinamide, *p*H 7.0; *b*, NAD$^+$, *p*H 6.0; *c*, N-ethyl maleimide, *p*H 6.0; *d*, acetoacetate, *p*H 9.2; *e*, Co^{2+}, *p*H 7.0 ; *f*, Cd^{2+}, *p*H 7.0.

active agents in biochemical electron transfer reactions has been indicated[1,2], and correlations between the redox potential of the quinones and their effectiveness in catalysing a particular type of phosphorylation reaction have been suggested[1,2,5]. This principle has never been demonstrated quantitatively by chemical kinetics.

The redox reactions of a number of organic free radicals against menaquinone as acceptor have been studied[6–10]. The rate constants k for electron transfer from some of these radicals to different acceptors as a function of the redox potential ($E^{\bullet 1}$) of the acceptors is the subject of the study described here. The fast-reaction technique of pulse radiolysis was used[10,11] for generating the free radicals in water (*a*) by reaction of OH radicals with some substrates (in the presence of N_2O to convert e^-_{aq} into OH radicals), (*b*) by reaction of e^-_{aq} with other substrates (in argon and ~1.0 M *t*-butyl alcohol to scavenge[9] the OH radicals).

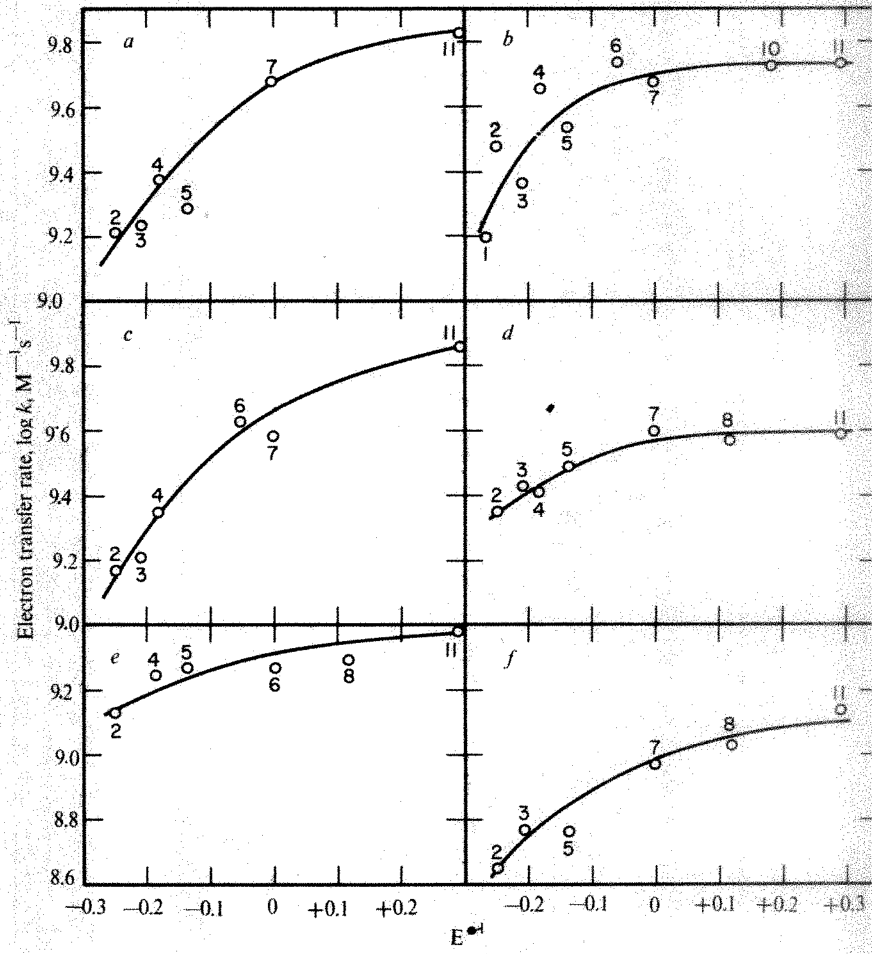

Fig. 2 Dependence of log k, the electron transfer rate constants of various free radicals (produced by reaction of substrate with OH radicals) on the $E^{\bullet 1}$ values of the acceptors. *a*, Formate, *p*H 7.0; *b*, isopropyl alcohol, *p*H 7.0; *c*, cytosine, *p*H 7.0; *d*, glycine, *p*H 8.0; *e*, triglycine, *p*H 10; *f*, glycollic acid, *p*H 7.0.

The acceptor concentration was $\sim 5 \times 10^{-5}$ M for quinones and $\sim 1 \times 10^{-4}$ M for other acceptors. The [radical] was kept at ≥5% [acceptors]. The k values were determined at ~22° C by following (*a*) the formation kinetics of the semiquinone radical anions of the quinones (at 400 nm) and of riboflavin (at 560 nm[13]); (*b*) the disappearance kinetics of MnO_4^- at 545 nm and of ferricyanide at 420 nm. From the pseudo-first order rates, the second order rates were determined. The following acceptors were studied: 9,10-anthraquinone (①, −0.266), 9,10-anthraquinone-2-sulphonate (②, −0.250), riboflavin (③, −0.208), 9,10-anthraquinone-2,6-disulphonate (④, −0.184), 2-hydroxy-1,4-naphthaquinone (⑤, −0.139), naphthaquinone-2-sulphonate (⑧, 0.118), 2,5-dimet benzoquinone (⑨, 0.176), MnO_4^- (⑩, 0.180), and *p*-b quinone (⑪, 0.293). The $E^{\bullet 1}$ values (*p*H = 7.0, 25° C) in parenthesis were from ref. 14.

The electron transfer reactions taking place can be repres as:

$$OH + RH_2 \rightarrow RH\cdot + H_2O \text{ and } e^-_{aq} + RH_2 \rightarrow RH_2^{\bar{\cdot}}\text{, follow}$$

$$RH\cdot + \rightarrow A^{\bar{\cdot}} + R + H^+$$

$$RH_2^{\bar{\cdot}} + A \rightarrow A + RH_2$$

The pK_a of the semiquinones[10,15,16] used lie in the 4·0–5.0: $AH\cdot \rightleftharpoons A^{\bar{\cdot}} + H^+$. The results are presented in Ta and Figs 1 and 2. In addition, the k values of CH

$(CH_3)_2\dot{C}OH$, $NH_2\dot{C}HCOO^-$ and Cd^+ to NAD^+ ($E^{\bullet 1} = -0$ as an acceptor were found to be 1.0×10^9, 1.0×10^9, 1.5 and 2.9×10^9 M^{-1} s^{-1}, respectively.

From the experimental results (rate values ±10%): quantitative correlation appears between the k values many free radicals and the $E^{\bullet 1}$ of various electron acce (quinones, riboflavin, NAD^+, ferricyanide and MnC (*b*) the rate is expected to increase with increase in between the donor radical and the acceptor (as indicate the different slopes for the various radical donors); (*c*) half-wave reduction potential $E^{red}_{1/2}$ of the radical is prob a better parameter to use but only limited data are availab there is a direct correlation, however, between $E^{\bullet 1}$ and (see Fig. 1), as well as between the electron affinity and (ref. 18); (*d*) as there is a good linear relationship betwee

Table 1 Date Constants of One-Electron Oxidation-reduction Reactions of Free Radicals with Various Acceptors in Aqueous Solution

Substrate	Donor radical	Rate of electron transfer, $k \times 10^{-9}$ M^{-1} s^{-1} * ① −0.266	② −0.250	③ −0.208	④ −0.184	⑤ −0.139	⑥ −0.055	⑦ +0.002	⑧ +0.118	⑨ +0.176	⑩ +0.180	⑪ +0.293
nate	$\cdot CO_2$ ‡	—	1.65	1.71	2.40	1.95	†	4.80	†	—	†	6.60
opyl cohol	$(CH_3)_2\dot{C}OH$ ‡	1.62	3.04	2.30	4.60	3.40	5.55	4.76	†	—	4.24	5.40
sine	$\cdot$C-OH ‡	—	1.48	1.61	2.26	†	4.29	3.80	†	—	†	7.20
ycine	$H_2{}^+$-Gly-Gly-$NH\dot{C}HOO^-$ ‡	—	1.36	—	1.79	1.88	—	1.88	1.97	—	—	2.46
ine	$NH_2\dot{C}HCOO^-$ ‡	—	2.25	2.72	2.56	3.12	†	4.00	3.30	—	†	3.90
ollic acid	$OH\dot{C}HCOO^-$ ‡	—	0.71	0.93	—	0.91	—	1.47	1.69	—	†	2.20
tinamide	$\cdot$NH §	3.50	3.80	—	—	†	—	5.40	5.45	7.25	—	7.00
)$^+$	$\cdot$NAD §	0.41	—	1.00	0.96	—	—	3.10	—	†	—	4.40
hyl aleimide	$\cdot NEM^-$ §	0.83	0.82	—	0.46	—	—	3.00	—	4.40	—	5.50
oacetate	$CH_3\dot{C}(O^-)CH_2COO^-$ §	0.67	2.10	†	0.72	1.53	0.73	3.70	†	3.34	4.80	†
alt sulphate	CO^+ §	1.05	†	2.55	†	†	†	4.10	6.83	†	†	7.35
nium lphate	Cd^+ §	1.03	†	5.10	†	3.57	†	4.68	7.35	†	7.80	7.70

* See text for acceptor corresponding to each number. $E^{\cdot 1}$(pH 7.0) used as the redox potential.
† Slow thermal reaction occurring in this system.
‡ Donor radical produced by reaction of substrate with OH radicals.
§ Donor radical produced by reaction of substrate with e_{aq}^-.

x potential and the energy of the highest occupied orbital[19] ulated by LCAO-MO method) one electron should be oved (or added) from that orbital in the rate determining

conclusion, these results show that, to a first approxima- the rate constants for electron transfer processes from radicals to various acceptors are proportional to the redox ntial of the acceptor. As redox reactions are used exten- y to introduce or withdraw electrons in biochemical tions, such correlations can be used to predict the course arious competitive reactions, as well as to derive an oximate $E^{\cdot 1}$ value for a compound.

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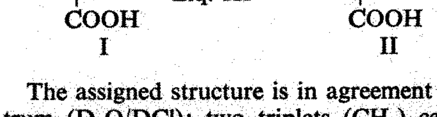
New Antibacterial Agent *via* Photofluorination of a Bacterial Cell Wall Constituent

We wish to report that a highly active antibacterial agent with novel properties, which is active against gram-negative as well as gram-positive bacteria, is generated by C-fluorination of a key component of the bacterial cell wall.

We proposed C-fluorination of bio-active molecules *via* photofluorination by fluoroxytrifluoromethane[1] for creation of agonists and antagonists of biologically important compounds[2]. This direct method of —CH→ —CF transformation appears successful in creating highly effective specific inhibitors of enzymes[3] *via* C-fluorination of properly selected bio-active molecules[1].

D-Alanine is a ubiquitous component of the bacterial cell wall[4]; it is endogenously synthesized by the bacteria, but, unlike L-alanine, it plays no essential role in human metabolism[5]. Further, the mechanism of antibacterial action of the penicillins, cephalosporins, and cycloserine has been shown to involve inhibition of various enzyme systems related to biosynthesis or utilization of the D-alanine component of the peptidoglycan of the bacterial cell wall[6–8]. Thus, it was thought that C-fluorination of D-alanine might generate a specific antimetabolite with antibacterial activity.

Photofluorination[1] of D-alanine with CF_3OF in liquid HF solvent produced 3-fluoro-D-alanine (II)—in better than 50% yield. Isolation was by ion-exchange chromatography on 'Dowex-X8' resin column, eluting with 1 M HCl. The compound was crystallized from water and had an m.p. of 168° C (dec.).



$$\underset{\text{I}}{H_2N{-}\overset{\overset{\displaystyle CH_3}{|}}{\underset{\underset{\displaystyle COOH}{|}}{C}}{-}H} \xrightarrow[\text{Liq. HF}]{CF_3OF;\ h\nu} \underset{\text{II}}{H_2N{-}\overset{\overset{\displaystyle CH_2F}{|}}{\underset{\underset{\displaystyle COOH}{|}}{C}}{-}H}$$

I II

[(S)-2-amino-3-fluoropropionic acid]

The assigned structure is in agreement with: (*a*) PMR spectrum (D_2O/DCl): two triplets (CH_2) centred at 5.3 p.p.m. (J_{H-F}: 60 Hz); two multiplets (CH) centred at 4.65 p.p.m. (5.02 and 4.28 p.p.m.). (*b*) F^{19} NMR spectrum (D_2O/DCl): two

Table 1 Summary of Effects of Test Drugs

Drug (µg ml^{-1})	Atria	Ventricles
Control	Exponential decay.	Exponential decay. Beating rate slower than atria and ceases sooner.
Tyramine 40	Overall depression of beating rate. Decrease in survival time.	Similar to controls.
Oxyfedrine 20	The curve swung to a more horizontal position. 0–8 d beating slowed. 9–19 d rate speeded up. Slight increase in survival time.	Curve swung to more horizontal position. Rate decreased up to day 4, rate increased from day 5–16. Survival time increased.
Oxyfedrine 40	Slight but uniform increase in rate from day 2 to day 15. No increase in survival time.	Very slight decrease in rate up to day 6, increase in rate from day 7 to 14. No increase in survival time.
Oxyfedrine Tyramine } 40	Similar to control; the two drugs cancelling each other out.	Similar to control. Drugs cancel.
Oxyfedrine 80	Similar to control; striking reduction in the variability of the rate. (Scatter.)	Uniform slight increase in rate. Reduction in scatter as for atria.
Isoprenaline 50	Moderate overall increase in rate but no increase in survival time.	Moderate overall increase in rate; no increase in survival. Greater scatter.
L-Noradrenaline 15	Similar to oxyfedrine 20. Curve swung to horizontal position. Decreased rate 0–8 d, increased rate days 9–23. Survival time increased more than by oxyfedrine 20.	No significant effect from day 0–5; increased rate from day 6–17. Increase in survival time.
Practolol 50	0–8 d similar control, 9–18 d rate increased. Slight increase in survival time.	Overall increase in rate starting slight and becoming large. Large increase in survival time.

The four hearts under each set of conditions represent a range of foetal ages, and therefore a range of developmental stages, giving a certain amount of variation in beating performance.

The performance of the youngest and oldest groups of hearts were examined separately, and predictably the youngest hearts lived longer and beat better than the oldest. The atrial and ventricular beating rates of the oldest control hearts were erratic in this preliminary series, and we suggest that for drug evaluation, hearts of less than 60% of term give more meaningful results.

Some interesting differential effects were demonstrated. Oxyfedrine, for example, at 20 µg ml^{-1} showed a positive effect on both atria and ventricles in the young hearts with no apparent effect on the beating of the older atria and ventricles; whereas isoprenaline affected both the young and old hearts equally.

As well as being a potentially useful assay for cardioactive drugs, it seems also that any substance which is to be given to pregnant women should be examined for its direct effect on the mouse foetal heart. Further study with human foetal heart should follow if any effect is demonstrated. It cannot be assumed, however, that the substance is harmless if there is no effect on mouse or human foetal heart; many drugs are not pharmacologically active in their pure form, whereas their breakdown products are. Complete drug evaluation will require cultures with each of the intermediate breakdown products at their likely concentrations. If after this screening a drug seems to have no effect, or even a beneficial one, it would

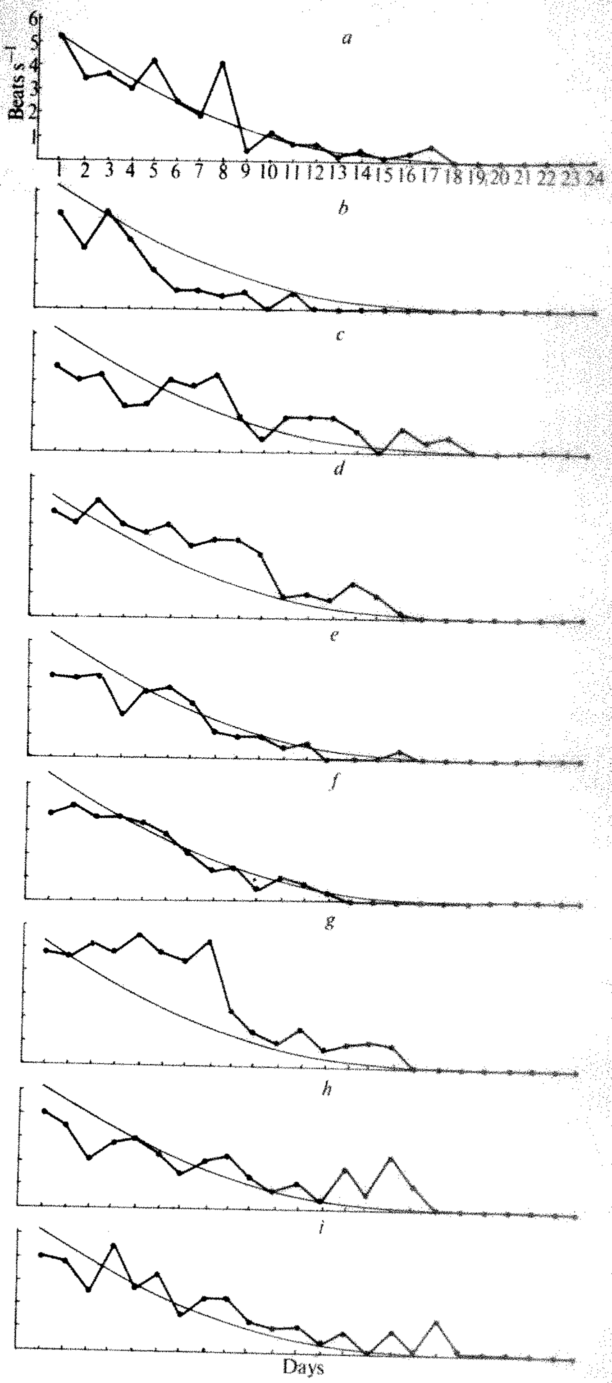


Fig. 1 Atrial rates of the control hearts compared with the atrial rates of the test hearts for the first 23 d of culture. Hearts from nine litter mates of comparable size were selected from pregnant mice for each set of experiments. (When one pregnant mouse contained only seven foetuses, two foetuses of the same crown rump size from another litter had to be used.) The experiment was repeated successfully four times, using a range of foetal ages 54% to 80% term (crown rump 9 to 16 mm). Hearts were cultured as previously described[5] on stainless steel mesh so that their lower surfaces were wetted by 65% medium 199 (Burroughs Wellcome), with antibiotics, 35% foetal calf serum, 50 µg ml^{-1} insulin, 0.1 µg ml^{-1} cortisol; in a water vapour saturated atmosphere of 95% oxygen and 5% CO_2 and at a temperature of 37° C±2° C. Six drugs were added to this standard medium to give the nine test media, as follows: (*a*) standard medium (control); (*b*) medium+tyramine hydrochloride 40 µg ml^{-1}; (*c*) medium+oxyfedrine 20 µg ml^{-1}; (*d*) medium+oxyfedrine 40 µg ml^{-1}; (*e*) medium+tyramine hydrochloride 40 µg ml^{-1} plus oxyfedrine 40 µg ml^{-1}; (*f*) medium+oxyfedrine 80 µg ml^{-1}; (*g*) medium + isoprenaline 50 µg ml^{-1}; (*h*) medium+L-noradrenaline 15 µg ml^{-1}; (*i*) medium+practolol 50 µg ml^{-1}. The medium was replaced every other day after observations had been made. The cultures were examined daily using a dissecting microscope, during which examination special efforts were made to maintain the same constant conditions of temperature, atmosphere and water vapour saturation.

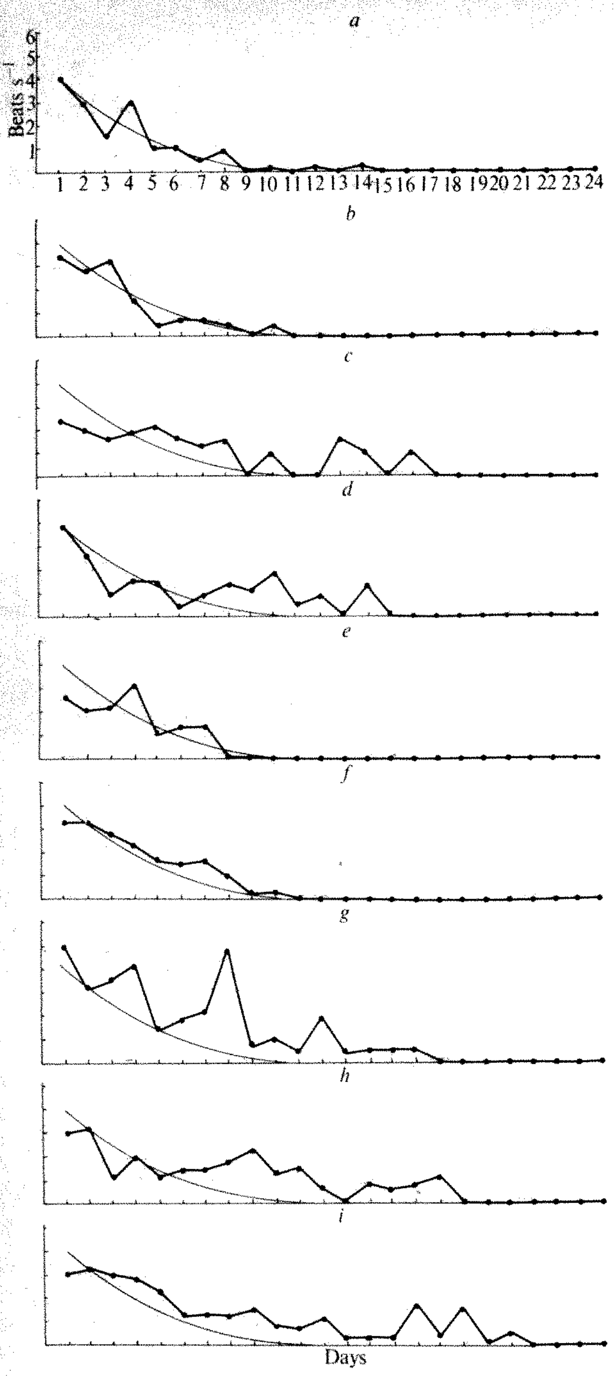


Fig. 2 Ventricular rates of the control hearts compared with the first 23 d for the test hearts.

seem reasonable to continue with further conventional clinical trials. If a new drug is depressant to the foetal myocardium above a certain concentration, it would be unwise to exceed this in a subsequent clinical trial. If the substance were to affect only young foetal hearts it might still be safe in late pregnancy.

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Penetration of Asbestos through the Digestive Tract of Rats

MESOTHELIOMAS of the pleura and peritoneum[1-3] have been linked with the inhalation of asbestos fibres. We have shown[4] that asbestos fibres are present in drinking water, beer, wine and other beverages. Asbestos fibres can penetrate the mucosa of the stomach and intestine[5] and Telischi and Rubenstone[6] have found asbestos material in a gastric carcinoma. Godwin and Jagatic[7], reporting on mesotheliomas of the pleura caused by asbestos, noted that asbestos particles were widely distributed in various tissues. They suggested that particles may migrate from the lung through the blood and lymphatic system to all parts of the body. In the light of these reports, we surmised that asbestos consumed orally can pass through the gut into the blood stream and accumulate in various tissues.

To determine if this is the case we injected a suspension of asbestos fibres into the stomachs of rats. The suspension was prepared by shaking 2.5 g of chrysotile asbestos (Johns Manville No. 7RFO2) in 500 ml of water in a graduated cylinder. The suspension was allowed to settle for 30 min and the top 250 ml, which contained about 9.4×10^9 (1 mg) fibres ml^{-1}, was drawn off. Most of these fibres are 0.2 µm–2.0 µm long (Fig. 1). To ensure that fibres passed through the digestive tract without rats inhaling any of them, the asbestos was introduced into the gastrointestinal tract by opening the abdomen under anaesthesia and injecting the fibres directly into the stomach. Two to 4 d later the rats were killed and blood and tissues analysed for asbestos. To ensure that any fibres on the rat hair could not contaminate the organs removed during surgery, the rats were first thoroughly vacuumed and washed with double distilled water and methanol. During the removal of tissues wet towels were placed over the animals and around the incisions. Uncontaminated blood was drawn from the orbital sinus using a capillary tube.

To avoid formation of excess ash, which interferes with examination of specimens under the electron microscope, the tissues were first solubilized with soluene (Packard Instrument Company); the fibres were then centrifuged down, washed with methanol and ashed as previously described[4]. The ash was taken up in 1 ml of distilled water which had been filtered through a 0.2 µm filter, and 5 µl of this mixture was dropped on hydrophilic carbon-coated electron microscope grids supported on a ring peg. The grids were dried in a closed glass container (to avoid air contamination) under a heat lamp and were examined at magnifications of 20,000 and 80,000 with an electron microscope. The presence of chrysotile asbestos fibres was verified by electron diffraction. They were counted and the numbers corrected for dilution to determine the number in the original 1 ml sample. Results of recovery of asbestos fibres from rats treated in this way are shown in Table 1.

Rats in group 1 were given 9.4×10^9 fibres (1 ml) and killed 2 d later; those in group 2 received 94×10^9 fibres and were killed 4 d later. No asbestos fibres were detected in the blood of non-injected rats (controls) but 4.65×10^6 g^{-1} (a statistically significant increase) were found in the first group.

This is the first time direct physical evidence for the presence of asbestos fibres in the blood has been obtained. Four days after treatment the amount of asbestos in the blood of rats in group 2 had dropped to 1.19×10^6 g^{-1} even though these rats received ten times as much asbestos. This indicates that blood

Table 1 Numbers of Asbestos Fibres Recovered from Treated and Non-Treated Rats

	Controls mean*		Group 1† mean*		Group 2‡ mean*	
Blood	0.00	—	4.65¶	1.02	1.19	0.77
Spleen	2.33	0.67§	4.11	0.49	3.45	1.20
Omentum	2.46	0.46	2.74	0.87	18.25	7.79
Heart	2.09	0.41	—	—	2.28	0.40
Brain	0.05	0.02	0.31‖	0.19	0.29**	0.11
Lungs	1.06	0.26	1.80	0.54	1.74	0.26

* Average of five rats in all cases except where noted. † Animals injected with 9.4×10^9 fibres and tissues examined 2 d later. ‡ Animals injected with 94×10^9 fibres and tissues examined 4 d later. § Standard error of the mean. ‖ Four rats only. ¶ $P < 0.01$; ** $P < 0.05$.

(The figures in the table are fibres $g^{-1} \times 10^{-6}$.)

can clear itself of the fibres. Tissues such as the spleen, heart and lung also seem to have, to a lesser extent, the ability to clear themselves of the asbestos fibres, as the figures obtained from rats in group 2 indicate. They are lower than those for group 1 rats even though they received a larger dose, but the tissues had longer to clear themselves. Indeed, recent work in our laboratory with radioactive asbestos indicated that some tissues, such as the lungs, can reduce their fibre content by half in 2 d. This clearing action could possibly have quite an effect on the numbers of fibres found in the liver and kidney of the rats but unfortunately, because of the considerable amount of ash present in these organs even after soluene treatment, we could not examine these specimens under the electron microscope.

Another factor may contribute to the higher fibre count obtained for the organs of rats in group 1 compared with animals in group 2, namely, that there were four times as many fibres in the blood of rats in group 1. The residual blood in the tissues on removal from the animal would of course contribute to the total fibre count of a particular tissue.

This factor could have created the anomaly in the fibre counts in brains. In the brains of treated rats this was about six times higher than in the controls, and the increase in fibre count is statistically significant for rats in group 2 but not, oddly, for rats in group 1 even though there were more fibres in these animals. The greater number of fibres per gram in the blood of rats in group 1 could be responsible for the larger standard error in the counts of rats in group 1 and therefore did not allow the values obtained to be significant.

The brain seems to have little ability to clear itself of asbestos fibres, but the omentum has even less. This tissue, which surrounds the small intestine, appeared to accumulate most asbestos. Although the asbestos fibre content of the omentums in rats in group 2 may seem rather high (one extreme count contributed to the high standard error) the omentum does not seem to respond as do the other tissues. As well as having little ability to clear itself, fibre accumulation in the omentum may take place much more slowly than it does in other tissues. As the omentum consists partly of peritoneum and partly adipose tissue, asbestos fibres may enter the peritoneum by direct penetration of the intestinal wall. Concerning the prolonged retention of asbestos fibres in the omentum in women asbestos workers, Keal[8] found a higher incidence of peritoneal cancer than of lung cancer.

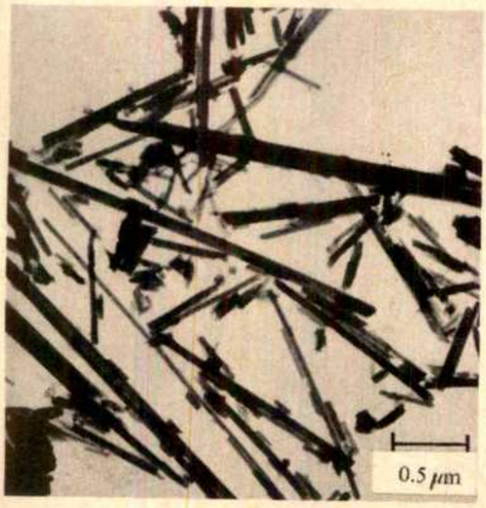

Fig. 1 Electron micrograph of the suspension of asbestos fibres injected into the rat stomach. Note the varying sizes of the bundles of fibres present.

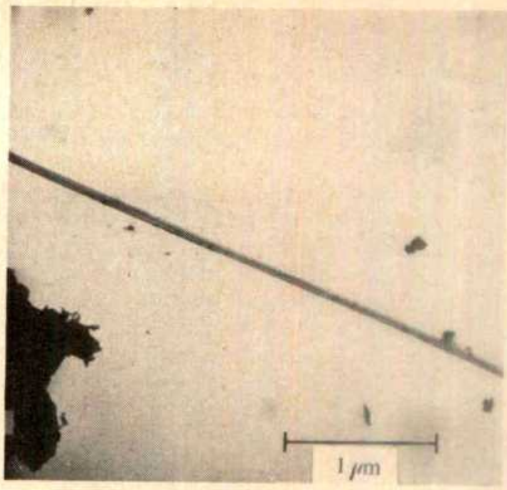

Fig. 2 Small bundle of asbestos fibres from the peritoneum of a rat. This bundle is at least 15 µm long.

The amount of asbestos found in the tissues of the controls is higher than we had anticipated, considering the age of the rats and the number of fibres they might have consumed in their drinking water[4]. But we do not know how much was in their feed or air. We have analysed the tissues of some people who died of natural causes and found somewhat similar levels. In one person there was twice as much asbestos in the brain as in the brains of our control rats and about one quarter as much in the omentum, but the omentum content was still higher than the brain.

Most of the fibres found were about 0.2–2.0 µm long but some measured 15 µm (Fig. 2) and one fibre in the blood of a rat was 23.55 µm long. We do not know how the fibres pass through the intestinal wall. Some long ones may pierce the gut like a needle, whereas pinocytosis may account for the absorption of the small ones.

We demonstrated earlier the presence of asbestos fibres in air, snow, city drinking water and a number of beverages[4]. The experiment described here shows that fibres of a similar size administered into the stomach of rats appear in the blood and accumulate in various tissues in the body.

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Presynaptic and Postsynaptic Effects of Lead at the Frog Neuromuscular Junction

Lead has long been known to be toxic to animals and humans, with some of its effects being attributable to actions on the neuromuscular system[1,2]. Since several other polyvalent cations have potent effects on neuromuscular transmission[3-19], the question arose as to whether one of the manifestations of lead toxicity might be an interference with neuromuscular transmission. Answers to this question might elucidate some mechanisms of lead toxicity and, at the same time, improve our understanding of junctional transmission. This report shows that lead influences both pre and postsynaptic events in neuromuscular transmission with the presynaptic ones being most sensitive.

There have been no previous electrophysiological studies of the effects of lead on neuromuscular transmission. However, Kostial and Vouk[20], in a study of the perfused superior cervical ganglion of the cat, found that lead nitrate, in concentrations as low as 12.1 μM, blocked ganglionic transmission. The authors concluded that this effect was due to a presynaptic action of lead because: (*a*) the acetylcholine (ACh) output of the eserinized ganglion, resulting from preganglionic nerve stimulation, was reduced by lead, and (*b*) the postganglionic response to perfused ACh was not affected by lead.

Experiments were performed *in vitro* on the isolated sciatic nerve-sartorius muscle preparation of the frog (*Rana pipiens*). Superficial neuromuscular junctions were located optically with a compound microscope (magnification ×400). Standard microelectrode and photographic techniques were used to monitor intracellular responses from individual end-plates. Responses were recorded first while the preparation was bathed in a control Ringer solution, then in the presence of lead, added as $PbCl_2$, and finally after the lead had been washed out. Solutions entered the experimental chamber by gravity and left by suction. The composition of the normal Ringer solution was: 111 mM NaCl, 2.5 mM KCl, 2.0 mM $CaCl_2$, 4 mM Tris-maleate. All Ringer solutions were maintained at *p*H 6.9 and at a temperature of 15° C. Preparations were exposed to lead by bathing them in a Ringer solution to which 0.01–0.10 mM $PbCl_2$ had been added. To record subthreshold end-plate potentials, nerve-muscle preparations were bathed in 0.8 mM Ca^{2+}/5.5 mM Mg^{2+}-Ringer solution[3,21]. Acetylcholine was applied directly to the end-plate receptors by iontophoresis from a micropipette[22,23] in order to test for postsynaptic effects of lead.

We have consistently observed that lead increases the frequency of miniature end-plate potentials (Figs 1 and 2). The records shown in Fig. 1*A* were made while the preparation was bathed in normal Ringer solution; those in Fig. 1*B* were made after bathing the preparation in 0.10 mM Pb^{2+}-Ringer solution for 5 min; and those in Fig. 1*C* were recorded after washing with normal Ringer solution for 5 min. Data taken from several fibres are shown graphically in Fig. 2 which is a log-log plot of the relationship between the lead concentration and the frequency of miniature end-plate potentials. The frequency during control conditions was 0.39 ± 0.20 s^{-1} (mean ± s.e.), and it increased by almost two orders of magnitude in the presence of 0.10 mM Pb^{2+}, the actual value being 22.1 ± 7.6 s^{-1}. When the fibres were washed with normal Ringer solution, the frequency returned to 0.42 ± 0.10 s^{-1} within 5–10 min. Thus, this effect of lead is reversible (see also Fig. 1*C*).

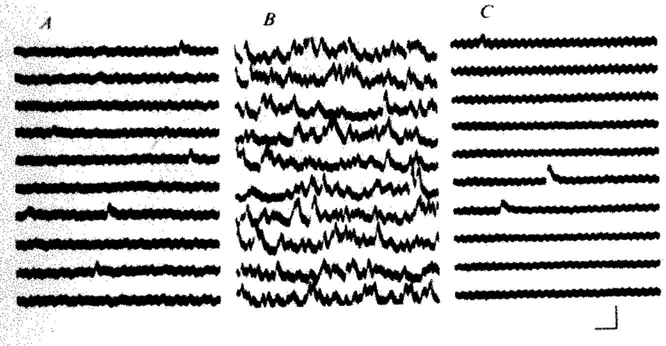

Fig. 1 Effect of lead on the frequency of miniature end-plate potentials. *A*, Control: the preparation was bathed in normal Ringer solution. *B*, Five minutes after exposing the preparation to 0.10 mM Pb^{2+}-Ringer solution: the frequency increased greatly while the amplitudes of the miniature end-plate potentials were essentially unchanged. *C*, Five minutes after washing the preparation with normal Ringer solution: the frequency was no longer elevated. Oscillograms *A–C* were taken from the same neuromuscular junction. Vertical calibration: 1 mV. Horizontal calibration: 50 ms.

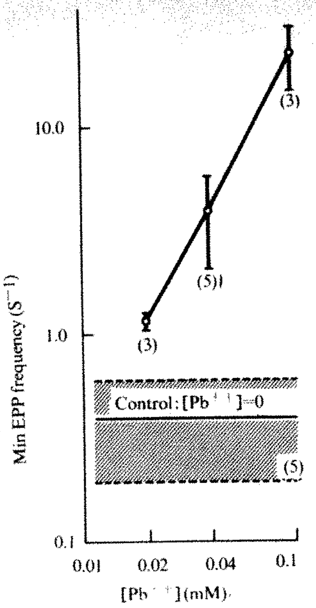

Fig. 2 A double logarithmic plot showing the dose-response relationship between the lead concentration and the frequency of miniature end-plate potentials. Each point is the mean ± s.e. for measurements made from three to five fibres. The control frequency was measured in the absence of lead and is depicted by horizontal lines (mean ± s.e.). All measurements were made after the preparations were exposed to the Pb^{2+}-Ringer solution for 5 min. Although not shown in the above plot, the mean frequency decreased to 0.42 ± 0.10 s^{-1} 5 min after washing the preparations with normal Ringer solution (see also Fig. 1*C*).

In 1954, del Castillo and Katz postulated that two modes of transmitter release exist at the frog neuromuscular junction—one for the spontaneous release of individual quanta and another for the synchronous or phasic release of several hundred quanta of the transmitter, ACh[11]. This hypothesis is based on the observation that magnesium did not reduce the frequency of the spontaneous release of transmitter even though this ion had been shown previously to inhibit the phasic release following a nerve impulse[3]. Calcium antagonized this blocking effect of magnesium[3], but it had no clear effect on the frequency of miniature end-plate potentials[24]. Our experiments show that lead depresses the phasic release of transmitter (Fig. 3). Subthreshold end-plate potentials were recorded in 0.8 mM Ca^{2+}/5.5 mM Mg^{2+}-Ringer solution. The motor nerve was stimulated with pairs of pulses to facilitate transmitter release during the second transmission, thereby producing easily detectable (facilitated) end-plate potentials. The interval between pulses in a pair was 5–7 ms, and the pair of stimuli were applied at a rate of 3/s. Since the amplitude of end-plate potentials recorded from preparations bathed in high magnesium and low calcium Ringer solution varies according to the quantal hypothesis[25], many recordings were made in a particular condition to obtain the mean ± s.e. of the end-plate potential. The facilitated end-plate potential during the control period was 4.71 ± 0.15 mV ($n = 18$) (Fig. 3*A*). The preparation was then exposed to 0.01 mM Pb^{2+}, the Ca^{2+}/Mg^{2+} ratio remaining unchanged; within 1 min, the facilitated end-plate potential decreased to 0.49±0.08 mV (n=21, three failures). Figure 3*B* shows a typical effect of 0.01 mM Pb^{2+}—the facilitated end-plate potential was reduced to a single quantal response while the unfacilitated one was blocked completely. As 0.10 mM Pb^{2+} does not block miniature end-plate potentials (Fig. 1*B*), the blocking effect on the end-plate potential occurred presynaptically by decreasing the quantal release of acetylcholine. When the nerve-muscle preparation was washed with the control Ringer solution, the facilitated end-plate potential recovered almost completely within 10 min to 3.97±0.10 mV (n=38) (Fig. 3*C*). Thus, this effect of lead is also reversible.

It is important to note that the subthreshold end-plate potential was reduced by 0.01 mM Pb^{2+} within less than 1 min whereas no significant increases in the frequency of miniature end-plate potentials were detected when this amount of lead was added to normal Ringer solution. Larger concentrations of lead were necessary to cause detectable increases in the frequency of the spontaneous release of transmitter (Figs 1 and 2). This dose differential, together with the two different effects of lead on transmitter release—stimulating its spontaneous release while inhibiting its phasic release—clearly indicate that these two processes are distinct. However, these processes may not be entirely independent[26].

To confirm our conclusion that lead reduced the end-plate potential by a presynaptic block and not by a postsynaptic one, other experiments were performed to see if lead decreased the ACh sensitivity of the postsynaptic (end-plate) membrane; this sensitivity was determined by dividing the magnitude of the ACh potential in mV by the total charge in nC that was delivered from the iontophoretic pipette[27]. The ACh sensitivity was not affected by 0.01 mM Pb^{2+}; however, 0.10 mM Pb^{2+}, a dose ten times that sufficient to reduce the end-plate potential in the experiments described above, was shown to have a weak curariform action (Fig. 4). Lead caused a small reduction (5.4%) in the ACh potential, which is the depolarization of the end-plate membrane following the iontophoretic application of ACh, when the tip of the ACh micropipette was very close to the receptors (Fig. 4*A* and *B*). In these conditions, the ACh sensitivity both in the absence and in the presence of lead was near 100 mV nC^{-1} (ref. 28) and the density of ACh-receptor complexes probably approached that which occurs following the neural release of ACh. When this density was decreased by moving the ACh micropipette away from the receptors, however, the ACh sensitivity was 5.3 mV nC^{-1} and fell to 0.9 mV nC^{-1} (65% reduction) in the presence of lead. Thus, lead can only block postsynaptically in conditions in which the density of ACh-receptor complexes is much lower than it is during an end-plate potential.

The action of lanthanum at the frog neuromuscular junction[9,13-17] is quite similar to the presynaptic effects of lead described in this report. Moreover, there are other ions, in addition to those of lead, lanthanum, and magnesium, which inhibit the phasic release of transmitter—namely, those of beryllium, manganese, nickel and zinc[3,9,13-19]. It is not known precisely with which ligands of the presynaptic membrane these heavy metals combine. Perhaps we shall eventually be able to relate a specific physico-chemical characteristic of these metal ions to their potency as blockers of presynaptic release of transmitter. If so, we might thereby gain some insight concerning the chemical nature of those ligands that are involved in excitation-secretion coupling.

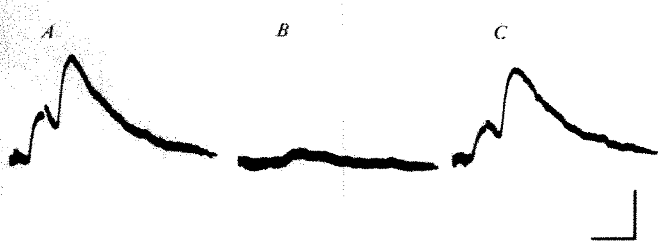

Fig. 3 Effect of lead on the end-plate potential. Neuromuscular transmission was blocked by bathing the nerve-muscle preparation in 0.8 mM Ca^{2+}/5.5 mM Mg^{2+}-Ringer solution, thereby making it possible to record subthreshold end-plate potentials. Facilitated end-plate potentials were recorded intracellularly by stimulating the motor nerve with double shocks at a rate of 3 s^{-1}. *A*, Responses to nerve stimulation while the preparation was bathed in the control Ringer solution. *B*, Effect of a 1 min exposure to 0.01 mM Pb^{2+} added to the modified Ringer solution. Notice that 0.01 mM Pb^{2+} blocked the unfacilitated end-plate potential and reduced the facilitated one. *C*, Responses 10 min after the preparation was washed with the control Ringer solution. The amplitudes of the facilitated end-plate potentials in *A*–*C* are similar to the mean values which are given in the text. Vertical calibration: 2 mV. Horizontal calibration: 10 ms.

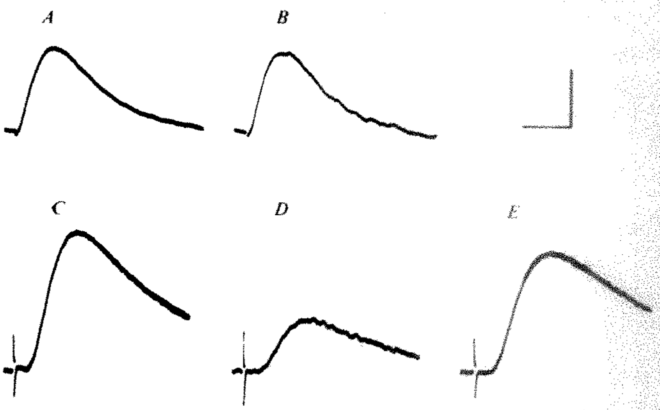

Fig. 4 Effect of lead on the amplitude of the ACh potential. *A*, ACh potential recorded from a neuromuscular junction bathed in normal Ringer solution. The tip of the ACh micropipette was optimally positioned at the end-plate receptors since the latency was very small (less than 1 ms) and the ACh sensitivity high (near 100 mV nC^{-1}). *B*, ACh potential recorded within 1 min after washing the preparation with 0.10 mM Pb^{2+}-Ringer solution; the iontophoretic current pulse and the position of the intracellular microelectrode and of the ACh micropipette were unchanged from *A*. *C*, ACh potential recorded several minutes later when the preparation was again bathed in normal Ringer solution. The ACh micropipette was moved away from the muscle fibre until a latency of 40 ms resulted. The ACh sensitivity was 5.3 mV nC^{-1}. *D*, ACh potential recorded within one minute after washing the preparation with 0.10 mM Pb^{2+}-Ringer solution. *E*, ACh potential recorded after washing the preparation with normal Ringer solution. The total charge delivered from the ACh micropipette and the position of the intracellular microelectrode and of the ACh micropipette were the same in *C*–*E*. Note that 0.10 mM Pb^{2+} significantly lowered the ACh potential in *D* whereas it lowered only very slightly the one in *B*. *A*–*E* were taken from the same end-plate. Records of the iontophoretic current are not present; only the iontophoretic artefacts appear on the voltage traces. The resting potential was −98 mV. Vertical calibration: 5 mV. Horizontal calibration: 20 ms in *A*–*B* and 100 ms in *C*–*D*.

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T Cell-dependent Mediator in the Immune Response

SUPERNATANTS of allogeneic lymphocyte cultures enable bone-marrow derived (lymphocytes) to respond to sheep red blood cells (SRBC) *in vitro*[1–3]. It was suggested that a non-specific T cell-dependent mediator may be involved in the normal immune response to this antigen. Indeed, T cells educated to one erythrocyte antigen can, in the presence of that antigen, greatly facilitate the response of B cells to other non-cross reacting erythrocytes[4,5]. T cells educated to protein antigens can also generate such a non-specific event in the interaction of T and B cells *in vitro*[6]. The following experiments were designed to establish whether in this syngeneic system the phenomenon was dependent on the production of soluble mediators after activation of T cells by protein antigen.

Mice of the CBA/Ca strain were used and were of the same sex in each experiment. As a source of B cells for culture we used the spleens of mice which had been thymectomized at 6 to 10 weeks of age, later irradiated and reconstituted with 4×10^6 syngeneic bone-marrow cells[6]. Educated T cells were obtained by using spleen cells of irradiated adult mice injected with 10^8 syngeneic thymocytes and 500 µg of keyhole limpet haemocyanin (KLH) (Calbiochem.) without added adjuvant. B cells alone respond poorly to SRBC *in vitro* and the facilitation of this response was used as an assay for T cell activity, using T cells educated to KLH. This assay system is designed to detect non-specific facilitation.

Three types of culture conditions were adopted. (*a*) Double chamber cultures based on the technique of Marbrooke[6] as modified by Feldmann and Basten[7] were used in the first experiments. They differed only in that culture chambers were smaller and were contained within 20 ml screw cap bottles. B cells (10^7) were cultured in the outer chamber which was bathed in 8 ml of culture medium. Educated T cells (5×10^6) were added to the inner chamber as required. The two chambers were separated by a 0.4 µm pore size 'Nuclepore' membrane. (*b*) For the "macro-assay" of supernatant material a culture system based on that described by Mishell and Dutton[8] was used, with the addition of nucleosides and mercaptoethanol as suggested by Click *et al.*[9]. B cells ($6–10 \times 10^6$) were cultured in 1 ml of medium. (*c*) To satisfy the need for a rapid and economical system to assay small amounts of supernatant, 6×10^5 B cells were cultured in Cooke's 'Microtiter' trays in a final volume of 100 µl of medium (Dutton, personal communication). T cell supernatants were prepared by incubating 10^7 KLH-educated T cells with 30 µg of KLH for 36 h in a 1 ml culture fluid volume. The supernatant was then collected, and the cells spun down. In the three culture systems the plaque-forming cell (PFC) responses to SRBC were assayed after 4 d in culture.

Table 1 Geometric Means (±s.d.) of the Plaque-forming Cell (PFC) Response to SRBC of Triplicate Cultures in a Modified Marbrooke Culture System

Inner chamber Antigen	Inner chamber Cells	Outer chamber Antigen	Outer chamber Cells	PFC/culture (s.d.)
(1) KLH	None	KLH and SRBC	T_{KLH}+B cells	196 (338–112)
(2) KLH	T_{KLH}	KLH and SRBC	B cells	207 (236–186)
(3) KLH	None	KLH and SRBC	B cells	38 (70–22)
(4) None	None	SRBC	T_{KLH} B cells	43 (80–24)

The system contained 1×10^7 B cells in the outer chamber and 5×10^6 T_{KLH} cells in the inner or outer chamber. KLH was added where indicated at a concentration of 30 µg ml^{-1} and 5×10^6 SRBC were added to the outer chamber of all cultures. T_{KLH}, T cells educated to KLH (see text).

Table 1 shows the result of an experiment in which KLH-educated T cells were cultured either together with (line 1) or separated from B cells by a 'Nuclepore' membrane (line 2). Facilitation of the B cell response occurred and was similar even when T cells were separated from B cells by the 'Nuclepore' membrane. Neither KLH alone nor KLH-educated T cells alone could produce this effect (lines 3 and 4). The supernatants from educated T cells cultured with KLH were subsequently tested on B cells in Petri dish cultures as described above (Table 2). It was found that a stable factor capable of generating non-specific facilitatory activity had been produced. Unprimed, passaged T cells could not generate such activity when cultured in the same way. These observations were also true in the microculture system, and Fig. 1 shows that the supernatant activity in such a system could be titrated. A "micro-assay" system of this nature makes it possible to attempt chemical characterization of the material. This supernatant activity is non-dialysable and can withstand freezing to −20° C. It is also possible to concentrate the activity 10-fold (by an Amicon Diaflow cell). We are now further characterizing this concentrated material.

The supernatant does not act by recruiting T cells specific for SRBC which may contaminate the B cells. Treatment of B cells before culture with anti-θ and guinea-pig complement[10] to remove any contaminating T cells did not prevent the B cell response to SRBC in the presence of the supernatant of educated T cells.

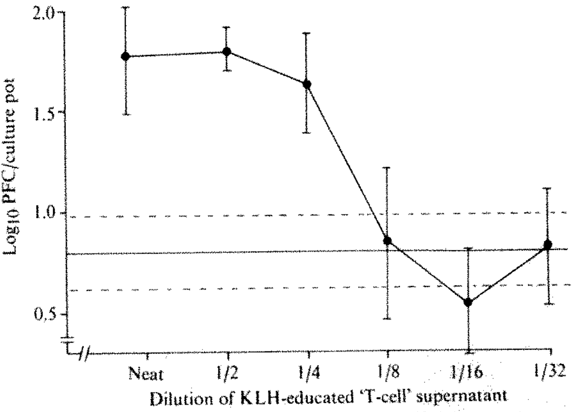

Fig. 1 Titration of the activity of T cell culture supernatant on the PFC responses of 6×10^5 B cells in microcultures. Each point represents the geometric mean of six replicate pots (with s.d.). Also shown is the effect of control medium (with s.d.).

Preliminary experiments designed to study the events involved in the production of this factor during the incubation *in vitro* showed that the addition of adherent cells to the cultures of

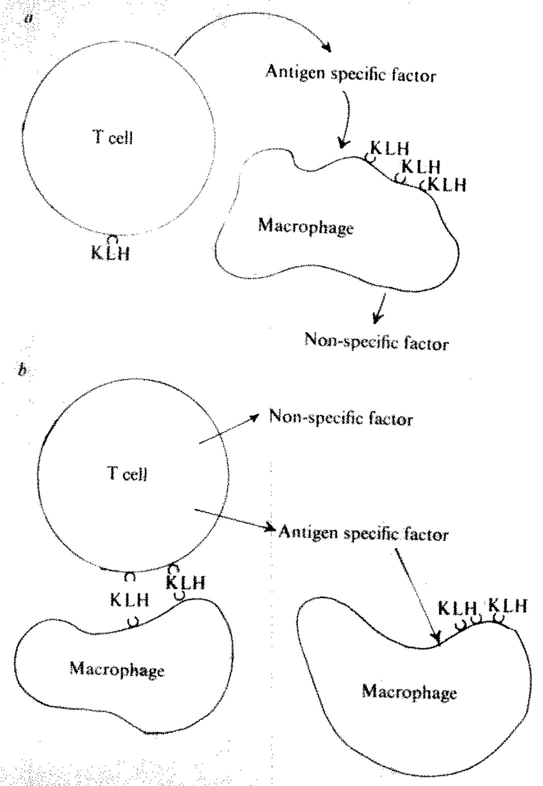

Fig. 2 Two possible mechanisms (*a* and *b*) for the involvement of adherent cells in the production of specific and non-specific factors by educated T cells.

educated T cells greatly enhanced the activity of the resulting supernatant (Table 3). In these experiments the adherent cells were prepared from B mice. It seems that, in the spleen cells from lethally irradiated mice reconstituted with thymocytes, there are insufficient functional macrophages to produce an optimal effect. It remains to be established whether the chemical nature of the active supernatant factor made with limiting numbers of adherent cells is identical to the supernatant factor made with excess adherent cells.

Table 2 Geometric Means (± s.d.) of the PFC Responses of Triplicate Cultures of 6×10^6 Cells to SRBC in the Presence of T Cell Supernatants

Treatment	PFC/ culture	Standard deviation
Supernatant from KLH-educated T cells incubated with KLH	765	738 794
Supernatant from "nil"-educated T cells incubated with KLH	94	79 112
Culture medium incubated for 36 h with KLH	67	47 98
2×10^6 KLH-educated T cells added directly to culture with KLH	2,822	2,344 3,388

"Nil"-educated T cells are taken from spleen of irradiated adult mice injected with 1×10^8 thymocytes and no antigen.

Table 3 Geometric Mean of the PFC Responses of Twelve Replicate Microcultures of 6×10^5 B Cells to SRBC in the Presence of "Supernatants" Prepared in Various Ways

Preparation of supernatant	PFC/culture pot Expt 1	Expt 2
Medium incubated with KLH	4	1
Educated T cells with KLH	16	25
Adherent cells with KLH	7	7
Adherent cells with educated T cells and KLH	95	90

Adherent cells required for the preparation of supernatants were obtained by incubating 6×10^6 spleen cells from B mice on Falcon Petri dishes, and after washing the monolayer thoroughly with medium for 1 h. Each culture contained 50 µl of the appropriate supernatant, 50 µl of B cells and 10 µl of SRBC containing 10^8 cells ml^{-1}.

In conclusion, our data demonstrate the generation of a supernatant factor by educated T cells in the presence of specific antigen, which can facilitate the response of B cells to a non-cross reacting erythrocyte antigen. We are unable to state whether the non-specific component of this phenomenon comes directly from T cells, or from other cell types or serum factors in the culture. The dramatic effect produced by the addition of adherent cells to T cell cultures suggests, however, that macrophages are intimately involved in its production, either directly or indirectly. Feldmann[11] has demonstrated that T cells produce a factor with immunological specificity which, when bound to macrophages and complexed with antigen, can induce B cells to respond specifically. Possible mechanisms for the involvement of adherent cells in the production of non-specific factor are (Fig. 2): (*a*) The production by KLH-stimulated T cells of a molecule of the type described by Feldmann which, when complexed with specific antigen, might activate macrophages to produce the non-specific factor. Indeed macrophages may release non-specific mitogenic substances when cultured in the presence of con A or endotoxin[12]. (*b*) Macrophages may be required to present antigen (KLH) to T cells for their optimal stimulation and subsequent production of non-specific and specific factors. These two models are not mutually exclusive.

If, however, macrophages are responsible for the production of non-specific factor, then the possibility must be considered, either that non-specific factor can provide the decisive signal directing a cell to induction, or that it may have a facilitatory role in lowering the threshold of triggering by latticed antigen. Such a factor may be an important component of adjuvant effect and may in part account for many experimental observations in which antigenic promotion or apparent lack of carrier specificity have been demonstrated[13-15].

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Depression of Freezing Point by Glycoproteins from an Antarctic Fish

SCHOLANDER and colleagues[1-3] observed that the blood sera of some polar fishes contain a substance of high molecular weight which lowers the freezing point. The general properties and structures of a family of several glycoproteins with this characteristic have recently been described in Antarctic fishes[4-14]. These "antifreeze" glycoproteins

(AFGP) consist of repeating units of the triglycopeptide Ala-Ala-Thr-*o*-disaccharide. Three active glycoproteins have been characterized; these differ only in polymer length, with molecular weights ranging from 10,500 to 21,000 g mol^{-1} as determined by ultracentrifugation, light scattering, or osmotic pressure[6,11]. According to the freezing point depression, however, the apparent molecular weight is only 20 g mol^{-1}, a value equivalent to >500 times the depression calculated from the molecular weights.

The blood sera of two species of Antarctic fish, *Trematomus borchgrevinki* and *Dissostichus mawsoni*, freeze at approximately −2.0° C, slightly below the temperature of the ice-salt water mixture in Antarctica[5]. Approximately one-third of the depression of the freezing point is caused by the AFGP, the remainder by dialysable substances of low molecular weight[5]. We have examined the rates of development of ice crystals and possible equilibria between ice crystals and aqueous phases, using differential thermal analysis (DTA) and direct microscopic observations of freezing and melting, respectively. The AFGP was purified from *T. borchgrevinki* serum as described previously[6,10].

The DTA experiments were carried out in aluminium vessels in a Mettler vacuum thermal analyser. Controls of water or solutions of 1% chicken ovomucoid[15] (a glycoprotein containing approximately 25% carbohydrate) in water and the solutions containing 1% AFGP froze at the same temperatures and had similar freezing and melting curves. Freezing of small volumes (10 λ) of such solutions usually occurred between −15 and −20° C, depending on the purity of the water and the experimental rates of lowering the temperature. The freezing temperatures in these conditions are usually considered to be related to the rates of nucleation and not to their freezing temperatures in equilibrium with ice. These experiments did not, therefore, indicate that the AFGP functions by inhibiting nucleation, that is, by inhibiting the initial formation of points of crystallization.

Direct observations of melting and freezing in volumes of approximately 50 λ at closely controlled temperatures were made with a Zeiss Universal polarization microscope (magnification 90) equipped with a photographic camera and a television screen monitor. The microscope slides were double-celled, with a compartment on each side of a centre divider. The whole slide was cooled by a slow flow of cold nitrogen gas and the temperature was maintained by heat supplied to the centre of the slide by a heating element in the divider. The thermocouple was Pt-PtRh, positioned in the centre of the slide. The apparatus was calibrated by determining the melting and freezing temperatures of pure water. A series of eight experiments was done and each experiment had five to twenty separate melting and thawing trials. Results of a typical experiment are summarized in Table 1.

Table 1 Freezing and Melting of Water and Solution of Antifreeze Glycoproteins in Water

Temperature adjustments	Observed changes In water containing ice crystals	 In water solution of 1% antifreeze glycoproteins containing ice crystals
° C phase		
0.0 holding	Melt and freeze	Crystals melt
−0.1 lowering	Frozen	Crystals do not melt, liquid does not freeze
−0.7 lowering	Frozen	
−0.8 holding	Frozen	Crystals grow, new crystals form until all solution frozen
−0.7 raising	Frozen	All frozen, no melting
−0.1 raising	Frozen	All frozen, no melting
0.0 holding	Melt and freeze	Melt

Water and antifreeze glycoprotein solution was initially frozen at −3° C and then allowed to melt at +0.1° C until 5–10% of solution remained as ice crystals. The temperature was then adjusted to 0.0° C and periodically lowered and then raised as indicated. The times at each temperature intermediate between freezing and melting were 5–10 min. All observations were made microscopically as described in the text.

In one series of trials, solutions of egg white ovomucoid were used in the control well of the microscope slide. The AFGP and the ovomucoid were both tested as 1% solutions in water. In all trials, both the melting and freezing point of the ovomucoid solution were −0.02±0.01° C. The melting point of an AFGP frozen solution or of ice crystals in AFGP solution was −0.01° C, and the freezing point of the solution containing ice crystals was −0.80° C. In another series of trials, a solution of AFGP containing a few crystals of ice froze at −0.78° C and melted at 0.00° C. After freezing, the sample was melted at +1° C until only a small bundle of crystals remained (approximately 0.1% of the solution had crystals). The sample was then adjusted to −0.60° C and held at this temperature for 300 min. No growth or melting of the crystals occurred. Similar observations were made when the FPDG solutions containing ice crystals were maintained at a temperature slightly less than the melting point (−0.10° C) or slightly more than the freezing point (−0.70° C).

From our experiments we conclude that: (1) Ice formed in a solution of AFGP seems to be normal ice—that is, it melts at 0° C. (2) Freezing and melting of AFGP solutions occur at rates similar to those at which water freezes and melts when equivalent amounts of heat are applied or removed at the respective melting or freezing temperatures. Thus there was no evidence indicating a comparatively rapid development of crystals in AFGP solutions as described by Hargens[16]. (3) It is not possible to prove a mechanism involving nucleation from the DTA experiments on kinetic effects. There are, therefore, no unusual "supercooling effects" as are commonly found in solutions in which initiation of freezing is very slow in the absence of crystals. The data indicate that there is no significant kinetic effect involved in the overall freezing mechanism.

The pronounced hysteresis and absence of equilibrium between the melting and freezing of solutions of AFGP are consistent with a mechanism which is not based on colligative properties. Models for the mechanism could include those postulating effects on either the structure or growth of ice crystals or the structure of water. If a model concerning ice is correct, the mechanism would most likely involve the development of ice crystals[10,17] after nucleation. If the model concerning the structure of water is correct, the mechanism could involve either the structuring of water itself or some intermediate state. The fact that ice crystals in AFGP solutions have normal melting points lends credence to the latter model.

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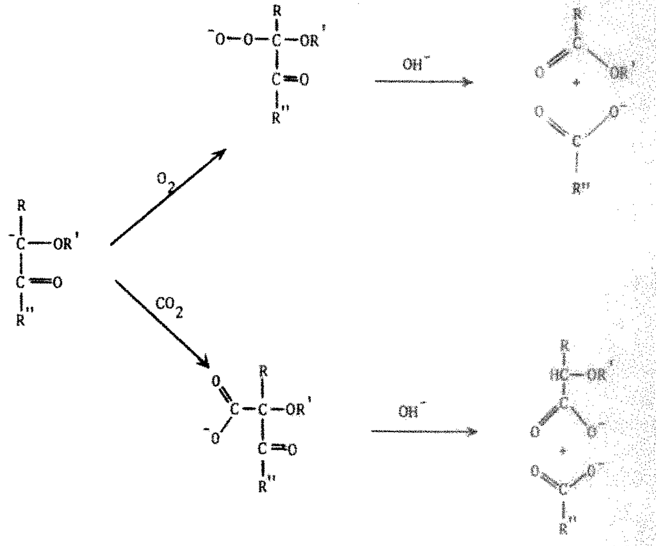
Plant Photorespiration—An Inevitable Consequence of the Existence of Atmospheric Oxygen

THE primary event in the glycollate pathway[1], the metabolic sequence responsible for the phenomenon of photorespiration in plants[2], is the oxygenation of ribulose 1,5-diphosphate (RuDP) to phosphoglycollate and 3-phosphoglycerate[3-5]. This reaction appears to be catalysed by the enzyme RuDP carboxylase[4]. When $^{18}O_2$ is supplied, incorporation of one atom of ^{18}O into the carboxyl group of one of the products only, namely phosphoglycollate, is observed. The other atom of the oxygen molecule is exchanged with the medium[5]. Similarly, intact spinach leaves incorporate isotopic oxygen, supplied as O_2, into the carboxyl groups of the glycollate-pathway intermediates, glycine and serine[6]. The purpose served by photorespiration has remained an enduring riddle[7]. Superficially, it would seem that oxygenation of RuDP must be deleterious to the photosynthetic process, necessitating the glycollate pathway to recoup as much of the phosphoglycollate carbon as possible with one quarter of it being lost as CO_2 at the glycine→serine conversion. Observations that net photosynthesis and plant growth in general are stimulated in oxygen-depleted atmospheres concomitantly with a suppression of photorespiration[8,9] reinforce this concept.

We suggest that oxygenation of RuDP, and therefore photorespiration, is chemically unavoidable when photosynthesis occurs in an oxygen-containing atmosphere because an intermediate generated during the reaction of RuDP carboxylase with RuDP is susceptible to attack by oxygen. We suggest that this intermediate involves the enolate anion of RuDP, the carbanion formed at C(2) being susceptible to attack by either CO_2 or oxygen. There is chemical precedent for both carboxylation and oxygenation of carbanions. For example, 2-methoxy-2-phenyl acetophenone reacts with both oxygen[10] and CO_2 (refs. 11, 12), presumably by means of the enolate anion, the structure of which resembles that of RuDP. The carboxylation of 2-methoxy-2-phenyl acetophenone has been suggested as a model reaction for RuDP carboxylase[12].

This hypothesis predicts that all RuDP carboxylases using the proposed mechanism, regardless of source, should also have oxygenase activity. If RuDP cannot be carboxylated other than as the enolate anion, then concomitant oxygenation is unavoidable if oxygen is present. If this is the case, attempts to reduce or eliminate photorespiration in crop plants by genetic manipulation are unlikely to succeed.

Because RuDP carboxylase has such an essential role in photosynthetic CO_2 fixation, this activity would have been conserved during evolution regardless of its inefficiency. Because the challenge presented by rising atmospheric oxygen concentration could not be met by modification of the carboxylase to prevent oxygenation, adequate means for the disposal of the phosphoglycollate waste product would have been required. In aquatic organisms dephosphorylation and excretion of glycollate may have been employed initially and many modern algal species still do this under certain conditions. A different mechanism would have been required, however, by terrestrial plants and we suggest that the glycollate pathway has evolved to fulfil this requirement.



In 2-methoxy-2-phenyl acetophenone R,R″ = C_6H_5, R′ = CH_3.
In ribulose, 1,5-diphosphate
R = $CH_2OPO_3^{2-}$, R′ = H, R″ = $CH(OH)CH_2OPO_3^{2-}$

Although plants which use the C_4 dicarboxylic acid (C_4) pathway do not exhibit the external manifestations of photorespiration[9,13], oxygen exchange studies indicate that photorespiration does occur[14] and it must be concluded that the CO_2 released is refixed before it can escape from the leaf. Thus C_4 plants do not constitute an exception to the present hypothesis. The rate of photorespiration in some C_4 pathway plants, however, as judged by the activities of glycollate-pathway enzymes, is probably less than that in plants with the Calvin cycle[15,16]. It has been suggested that this is because C_4 plants concentrate CO_2 at the site of RuDP carboxylase, thus allowing carboxylation to compete more effectively with oxygenation for the available RuDP[3]. The demonstration of an intermediate internal pool of CO_2 in C_4 plants[17] supports this idea.

A similar situation may occur in plants with crassulacean acid metabolism (CAM) where deacidification in the light is accompanied by release of CO_2 from malic acid[18]. As the stomata are closed at this stage of the diurnal cycle, the CO_2 concentration in the leaf may rise considerably above ambient, thus achieving the same stimulation of carboxylation at the expense of oxygenation that is suggested for C_4 plants.

The metabolic modifications of both the C_4 pathway and CAM might therefore be viewed, at least in part, as adaptations aimed at minimizing the inevitable deleterious effects of atmospheric oxygen on the photosynthetic process.

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Interspecific Hybridization in *Cardium*

BOYDEN[1] has shown that cross-fertilization takes place between the cockles *Cardium edule*, Linné, and *Cardium glaucum*, Bruguière. He used, as the criteria for successful hybridization, either the production of rotating trochophores, still within the confines of the egg sheath, or free-swimming veligers; no attempts were made to rear the larvae.

Galtsoff and Smith[2] were able to cross-fertilize two oyster species, *Crassostrea virginica* and *Crassostrea gigas*, and obtained what appeared to be normal, healthy veliger larvae. Davis[3] also successfully cross-fertilized these two species of *Crassostrea*, but although the larvae appeared normal and vigorous in behaviour, none survived beyond the tenth day of development.

A recently developed method of reliably rearing the larvae of *C. edule* and *C. glaucum* through to metamorphosis has enabled fully controlled cross-fertilizations to be conducted in the laboratory. This allows not only the determination of the success rate of cross-fertilization, but also the ability of the resultant larvae to survive.

Unfertilized ova and spermatozoa were obtained by inducing isolated, sexually mature cockles of both species to spawn in the laboratory. The experimental cross-fertilizations were carried out in 250 ml tall-form beakers, in which 5,000 to 8,000 ova were inseminated, using 1.5 ml of spermatozoa suspension prepared by diluting the entire produce of a single male in 500 ml of sterile seawater. The ova used in all the experiments were the results of normal spawning and in no cases were artificially stripped ova used. Insemination was effected as soon after discharge as possible, thereby minimizing post-spawning deterioration effects.

Each experiment consisted of duplicate cultures—one pair with the intraspecific cross, one pair with the interspecific cross and one pair of control cultures in which no spermatozoa were added to the ova. Maintenance of control cultures of uninseminated ova was essential for two reasons. First, although the seawater used in the test cultures had been carefully membrane filtered, it was impractical to keep the adult stocks in water thus treated, so there was always the possibility of fertilization by unwanted spermatozoa already present in the water. Second, the occurrence of hermaphroditic individuals is not unknown so the possibility of self-fertilization could not be overlooked.

After incubation for 24–48 h, during which time the fertilized ova had undergone sufficient development to be easily recognized, 100–200 eggs were removed and examined to determine the numbers of fertilized and unfertilized eggs. Further development was then allowed to take place, the cultures being maintained in optimum conditions for growth.

The results of these cross-fertilization experiments are shown in Table 1. It becomes immediately apparent from these figures that cross-fertilization is possible between *C. edule* and *C. glaucum*, further substantiating the findings of Boyden[1]. Examination of the results, however, reveals that the success rate of the interspecific crosses is small when compared with the intraspecific fertilizations. Subsequent rearing of the hybrid larvae met with little success in most of the attempted crosses; in a single case, however (experiment 15), the larvae lived long enough to metamorphose into adults. In general, cross-fertilizations between species originating from a mixed population (for example, the River Crouch) tended to be less successful than crosses between species from different geographic locations. The poor survival of hybrid larvae is of particular significance, especially in mixed cockle populations where it could result in the reduction of the reproductive capability of the two component species.

Table 1 Percentage Success of Cross-fertilizations between Individuals Originating from: *a*, Geographically Distinct Populations; *b*, a Mixed Cockle Population

a	Experiment												
Cross	1	2	3	4	5	6	7	8	9	10	11	14	Further development
Intraspecific	75	35	90	2	72	24	50	10	85	40	70	96	Majority to metamorphosis
Interspecific	8	5	10	0	<1	7	<1	0	20	0	15	0	All died
Control	0	0	0	0	0	0	0	0	0	0	0	0	—

b	Experiment						
Cross	9	10	11	12	13	15	16	Further development
Intraspecific	85	40	70	51	17	71	18	Majority to metamorphosis
Interspecific	12	0	0	0	0	30	4	Those of experiment 15 metamorphosed
Control	0	0	0	0	0	0	0	—

Female parent of interspecific crosses: experiments 1, 2, 5 and 6, *C. edule*; remaining experiments, *C. glaucum*.
Origin of individuals used in the experiments: *C. edule*, Shoeburyness, Essex; Ellanore, Chichester Harbour, W. Sussex; River Crouch, Essex; *C. glaucum*, Widewater, W. Sussex; River Crouch, Essex.

In mixed populations, individuals bearing apparent intermediate morphological characteristics do occur. Although limited, the successful rearing of hybrid larvae suggests that these intermediates are in fact true hybrids, and that their morphology is determined genetically and not simply as the result of environmental influence.

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BOOK REVIEWS

Bayesian Psychology

In Defence of Empirical Psychology. By Donald E. Broadbent. Pp. ix+221. (Methuen: London, February 1973.) £2.90.

On the wall of a colleague's laboratory there is a sign which reads "My mind is made up. Please don't confuse me with facts". In this book, which contains the six William James Lectures given by Donald Broadbent at Harvard University in 1971 along with four other lectures given from 1965 to 1970, the subtext seems to be—my mind is made up, don't confuse me with rational or theoretical argument. The common theme of the lectures is that facts must reign supreme in science and in society. The empirical method is the surest way to truth and understanding, and the empiricist's epistemology and his *weltanschauung* are in the highest tradition of Anglo-Saxon history and culture.

Broadbent argues that a person's scientific, political, moral and social lives are all of a piece. The way one goes about one's intellectual business is consistent with the way one goes about one's various other pursuits. Empiricists of Broadbent's stripe are different (and by implication better?) sorts of people than are rationalists, not only as scientists, but as social and political animals as well. One wonders what sorts of people theoretical mathematicians are.

The true empiricist seems to be one who subscribes wholeheartedly to a Bayesian view of life. As a scientist, the best strategy is to consider, at all times, the relative weight of evidence for alternative theories rather than to seek evidence in support of one favoured theory. As humans, Bayes's theorem seems to characterize much of human involuntary behaviour, particularly decision-making under conditions of uncertainty, and Broadbent argues that ". . . our voluntary behaviour ought to conform to . . ." the theorem as well (page 11). The theorem itself, proposed by the Reverend Thomas Bayes in 1736, simply provides a mathematical procedure for calculating the likelihood that one of several alternative hypotheses is correct. Broadbent's Bayesian outlook on life is that one should always consider the relative weight of evidence for at least two alternatives, be they courses of action, policy decisions or scientific hypotheses. Put crudely, one should try to calculate the odds that any particular alternative is correct relative to other alternatives, and then bet the odds. Query—is this strategy peculiar to empiricists, and do all empiricists adopt this strategy either as a way of doing science or of living in the world?

Broadbent implies that this is so, and characterizes his own work, and his life as well, as exemplifying this philosophy. Occasionally, as in his critique of some theoretical linguists, he endows proponents of opposing views with certain qualities inferred from his personal model of human personality . . . if someone is not an empiricist in his work, then he tends to be polemical and emotional about his work, and in other aspects of his life as well. For example, anti-empiricists (and only anti-empiricists) argue that it is important for one's work to be relevant to broad social issues (rather than to the design of, say, aircraft instruments), and their attitudes are seen as an imminent danger to society as a whole. All in all, this line of argument strikes me as unnecessarily *ad hominem*. The substantive issues in cognitive psychology and Broadbent's views on those issues can be treated far more convincingly without appeals to the good, the true and the beautiful.

Fortunately, Broadbent's own work does not conform to the extreme empiricist ideals he would have us embrace. He refers to his taking up a major portion of his Myers lecture by conceptual analysis rather than by experimental results as a "horrible example" (page 207). Nevertheless, his principal contribution to psychology has been conceptual. His most influential book, *Perception and Communication*, is only trivially empirical. Its impact, which in no small way helped to bring about the cognitive revolution in psychology, derived primarily from his compelling conceptual analysis of the brain as a processor of information, not from a set of compelling "facts" and data. Indeed, the predominant reaction of many stimulus–response behaviourists at the time was that the book was hopelessly mentalistic.

In short, the book reveals much of Broadbent the person as well as Broadbent the scientist, and the two are not as congruent as he seems to believe. But then, none of us is as self-consistent or rational as we tend to feel we are. To those of us involved in experimental psychology this book provides an interesting and sometimes revealing picture of one of our more distinguished colleagues. To those contemplating a career in science, I would add this note of caution: do as Broadbent does, not as he says he does. Sam Glucksberg

Air–Sea Interface

Aimosphere–Ocean Interaction. By E. B. Kraus. Pp. viii+275. (Clarendon: Oxford; Oxford University: London, April 1972.) £7.50.

This book has the avowed purpose of telling meteorologists something about relevant studies in oceanography and *vice versa* and also of interesting physicists in the physics of the environment. There can be no doubt that both these tasks need undertaking and the author's contribution to this end is to be welcomed. The book opens with a condensed review of the fluid mechanics needed for treating the dynamics of large-scale flows. This is a useful recapitulation of the subject and serves as a source of reference to the relevant equations for the rest of the book. Following this, the physical scene is set with an account of the properties of sea water on one side of the air–sea interface and of moist air on the other, with the transfer processes between them. A chapter on radiation gives a clear and up-to-date summary of the vicissitudes of solar radiation, in its passage through the atmosphere and into the surface layer of the sea, and also of terrestrial radiation.

The subject of surface waves has a long history of investigation and a voluminous literature but it is one in which important advances are still being made. The chapter on this subject gives an account of recent theories of wave generation by wind and an interesting feature is the attention paid to the properties of the flow in the air over waves as well as in the water. The next two chapters deal with turbulent transfer near the interface, in which the coupled transports of heat and moisture in relation to that of momentum and energy are of particular concern, and processes in the planetary boundary layers where Coriolis effects become important. A parallel treatment of effects in both the atmospheric and oceanic layers is followed as far as possible.

Three-dimensional interactions form the subject of the last, and longest, chapter and in a sense the whole book has been preparing the ground for this wide field of study. The interrelation of sea-surface temperature and the dynamics of the tropical atmosphere, the development of hurricanes and the inter-tropical convergence zone are among the topics treated. The properties of inertio-gravity and planetary waves in the ocean are discussed in relation to the response of the ocean to storms and to seasonal changes in the wind regime. It will be seen that the appearance of this book is particularly opportune at a time when GARP (the Global Atmospheric Research Programme) is getting into its stride and GATE (GARP Atlantic Tropical Experiment) is in active preparation.

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Coastal Habitats

Ecology of Salt Marshes and Sand Dunes. By D. S. Ranwell. Pp. xiv+258+16 plates. (Chapman and Hall: London, December 1972.) £4.60.

Since the emergence of ecology as a distinct discipline, both sand dunes and salt marshes have exercised an almost magnetic attraction for plant ecologists. It is not difficult to recognize the cause of this attraction. Both types of habitat have very distinctive types of vegetation which contain a high proportion of species restricted to them, and many of these species show obvious adaptation to the special conditions which prevail. On salt marshes, particular attention has been given to the causes of zonation and to the adaptation of plants to salinity and to periodic immersion in seawater, while on sand dunes interest has generally been focused on adaptation to movement of sand and on mineral nutrition. It has been generally accepted that both habitats provide clear evidence of vegetational succession and indeed sand dunes were among the habitats where this process was first recognized and described in the early years of this century.

It has long been recognized that a full understanding of both habitats requires knowledge of coastal physiographic processes and it now seems likely that the establishment of flowering plants on freshly deposited marine sediment or wind-blown sand is dependent on the activities of the soil fauna and of microorganisms.

In recent years, both habitats have acquired a less welcome reason for attracting the attention of ecologists. This is the vulnerability of salt marshes to pollution of estuarine and coastal waters and of sand dunes to destructive erosion apparently activated simply by the wearing away of their vegetation by holiday-makers.

The success of interdisciplinary investigations of both habitats, and the recognition of their vulnerability to damage, fully justify a new appraisal of the state of our knowledge of their overall ecology. Dr Ranwell has set out to do this. Certainly a lot of information is presented but often in the style of a summary without any real synthesis. For example, the description of physiographic types of dune-systems is written almost in the form of notes. Maps and diagrams would allow a reader, unfamiliar with more than a few sites, to grasp the full range of diversity and its probable causes. One of the four sections of the book is devoted to the effects of man and draws attention to many aspects of the ecology of dunes and salt marshes which were either unrecognized, or regarded as less important, in accounts written at a time when damage was less obvious and conservation was not uppermost in our minds.

Although the title of the book includes the unqualified word ecology, the treatment of both animals and microorganisms is brief and superficial. Rabbits, once so important, are discussed in a few short paragraphs and there is almost no mention of birds. The few pages devoted to insects are largely concerned with energy-flow, not with their biology, and other groups of invertebrates are scarcely mentioned. There are scattered references to microorganisms but such interesting groups as the bacteria which are concerned with oxidation–reduction processes in waterlogged soils of salt marshes are not discussed.

The book is primarily concerned with plant ecology but, even in this, the author deliberately restricts himself in a way which seems to lead to a confused presentation. The overall successional relationships are omitted on the grounds that they are often an oversimplification. But this is true of most ecological generalizations and not least of attempts to formulate flow diagrams of energy or substances through ecosystems. These are generally based on very sparse measurements of fluxes and inadequate knowledge of pathways. Then all descriptions of the composition of vegetation are excluded on the doubtful assertion that "the presence or absence of most species in a habitat is irrelevant to the great majority of other species (including man) in the habitat". One must wonder what does control the composition of vegetation and the population dynamics of animals. As a consequence of these two omissions much of the discussion and analysis has no clear focus. It is not easy to reconcile this rejection of accurate description with the plea on page 181 to give more attention to detailed studies of parts of ecosystems in order to understand the whole.

Much of the book seems symptomatic of a deep seated confusion in the minds of many ecologists. Description may not bring understanding but at least it defines what has to be understood. Analysis and many numerical data derived from analysis are often still purely descriptive. What is a model but a form of description? The equation on page 84 which is claimed to be a model of accretion and has its constants given to three significant figures, but fails to give the units of measurement, shows convincingly that numbers alone do not improve bad science.

C. D. Pigott

Food Technology

Health and Food. Edited by G. G. Birch, L. F. Green and L. G. Plaskett. Pp xi+224. (Applied Science: Barking, Essex, 1972.) £4.20.

This is a collection of papers read at a meeting organized by the authors as one of a series of annual international symposia of the National College of Food Technology, University of Reading.

It was intended as a discussion of the potential health problems that may arise from changes taking place in our food because of the rapid development of food technology. Almost all our food—a figure of 98% of the total energy intake has been suggested—passes through the hands of the food processor. Two questions were to be asked: are manufactured foods safe from the point of view of additives, processing changes, pesticide residues and so on; and what should be regarded as a good diet?

As might be expected, no conclusions were reached but the topics covered included discussions of consumer attitudes to processing, hazards of natural foods, methods of testing additives, legal aspects, pesticide residues in baby foods, yeast protein, ready-made meals, clinically designed foods, space foods, diet and longevity, problems arising with new foods. Speakers were drawn from industry and the academic field and from the United States, Holland, Poland and France as well as the United Kingdom.

As the titles indicate, the symposium was somewhat diffuse and unstructured but the material including the discussions provides useful material for continuing discussion and argument.

A. E. Bender

Phase Transitions

Phase Transitions and Critical Phenomena. Edited by C. Domb and M. S. Green. Volume 1. *Exact Results.* Pp. xv +506. (Academic: London and New York, December 1972.) £10; $31.

This is the first in a projected series of at least four volumes on the theory of phase transitions, intended to serve as a standard reference text for some time to come, particularly for graduate students. This intention sounds a little like trying to catch a rainbow, so rapidly is knowledge advancing in this area, but the book can stand on its merit as a set of high-quality review articles.

The book begins with two prefaces (one to the series, one to this volume) by the editors, and an introductory note by C. N. Yang. In only a few pages, they give a good overall view of the present state of the subject, how it got there, and how it might conceivably develop in the future.

The first of the review articles, by R. B. Griffiths, is about rigorous results and theorems. As we have come to expect from this author, it is a model of clarity. It also reveals his sure touch in dealing with the perennial dilemma facing all authors of such articles: whether to risk wearing out the reader by including too much mathematical detail, or risk losing conviction and coherence by not including enough. Connoisseurs will also enjoy this article for some entertaining misprints, such as "by purring bounds on the magnitude of the interaction energy" on page 24, and a real collector's item (too complicated to quote here) in equation (2.C2) on page 19.

There follow four shorter articles. First, J. Ginibre supplies some of the mathematical detail omitted from Griffiths's article, giving a clearly written and fairly complete account of the proof that infinite-volume correlation functions exist and are analytic for sufficiently dilute quantum systems. Next, G. G. Emch describes the C*-algebraic approach to phase transitions. This approach uses a great deal of special mathematical formalism, and it is often difficult for the uninitiated to understand either the meaning of the definitions and theorems or what purpose they serve. The great virtue of this article over most articles in this field is the effort that has gone into explaining these very points.

The next article, by C. J. Thompson, treates one-dimensional models with short-range interactions. Such systems never show phase transitions, but they do fit the secondary theme of this volume, exact results. The fourth article of this group, by H. N. V. Temperley, surveys the methods that have been devised for calculating exactly the thermodynamic properties of the two-dimensional Ising model (two-state "atoms" on each vertex of an infinite square lattice). Their variety and interest are a testimony to the vitality of this problem, first solved by Onsager (1944) in a *tour de force* of mathematical technique.

A slightly longer article, by I. Syozi, is a *tour de force* of a different kind; it shows how Ising models on an astonishing variety of two-dimensional lattices can be solved by transformations and extensions of Onsager's result. The most picturesquely named lattice is the "Asanoha, or hemp-leaf", lattice; the physically most surprising result is that some of the more complicated lattices treated can have three or even five transition temperatures.

The last article, by E. Lieb and F. Y. Wu, with insertions by E. Barouch, D. B. Abraham and R. J. Baxter, is almost a book in itself. It deals with the many variants and generalizations of the "two-dimensional square ice" model first solved by Lieb in 1967. Unlike the others in the book, this is an article for the specialist, going into great mathematical detail and presenting many results that seem to be new.

The volume can be strongly recommended to anyone with £10 to spare and an interest in recent research in the theory of phase transitions.

O. Penrose

Semiconductors

Theory of Electrical Transport Semiconductors. By B. R. Nag. Pp. vii+227. (Pergamon: Oxford and New York, December 1972.) £4.50.

The theory of electron transport in semiconductors has made great strides forward over the last six or seven years. Monte Carlo and iterative computational methods have been developed and successfully applied to a large number of semiconductor problems. Most researchers feel that the way forward lies in the exploitation of these methods. It is to be regretted that Professor Nag is not of that company. The new methods are dismissed in a few lines on the last page of the text with the implication that they are inferior to approximate analytic methods and do not help to build up physical insight. It would be more correct to say that the new computation procedures provide exact solutions to the Boltzmann equation and leave the researcher free to concentrate on the physical processes involved. Moreover, it is hard to imagine a calculation more replete with shafts of physical insight than a Monte Carlo simulation which follows individual electron trajectories.

The book under review is concerned with electrical conductivity theory as it existed before the introduction of modern computational methods. Electron states are discussed in an elementary way in chapter 1 and phonons and electron-phonon interactions are similarly treated at the beginning of chapter 2. The last twenty pages of chapter 2 provide a useful summary of the significant scattering processes in semiconductors. The bulk of chapter 3 is concerned with the relaxation time approximation and the relaxation times for the various scattering mechanisms are calculated in chapter 4. Experimental techniques are briefly reviewed in chapters 5 and 6 and chapter 7 is devoted to the application of semi-analytic techniques to high-field transport problems.

The balance of the book is poor. Considerable space is devoted to elementary notions concerning electrons and phonons which are adequately treated in a number of well known texts. Basic transport theory, on the other hand, is skimmed over very lightly. No discussion is given of either thermal transport phenomena or the Onsager relations. Boltzmann's equation is never stated properly: both the time derivative of the distribution function and Liouville's theorem are omitted from the derivation of the equation and there is no discussion of the limits of its validity. Variational methods for solving the linearized Boltzmann equation are given just two pages of text with no derivation or discussion of the functional involved. There are also a number of worrying errors in the text. The first examples appear on page two with the statements that the momentum of an electron is given by $-i\hbar\nabla\psi$ and its energy is $i\hbar\partial\psi/\partial t$.

The book is aimed at graduate students and new entrants to the field of semiconductor transport theory. There is certainly scope for a new book for this audience which carefully presents the basic theory, deals with the recent advances and points the way forward. The text under review does none of these things and is altogether too slipshod to be recommended.

P. N. Butcher

Antibiotic Action

The Molecular Basis of Antibiotic Action. By E. F. Gale, E. Cundliffe, P. E. Reynolds, M. H. Richmond and M. J. Waring. Pp. xviii+456. (Wiley: London and New York, 1972.) £8.

This book deals mainly with studies on the biochemical and molecular basis of antibiotic action. As indicated by the authors in the preface, however, the book is also concerned with other drugs and compounds whose cellular targets are similar or somehow related to those of the antibiotics.

The volume is divided into eight chapters beginning with a brief outline of the Ehrlich concept of chemotherapy including a short account of the development and mode of action of arsenical drugs, sulphonamides and early work on antimetabolites. There is a brief description of the biochemical targets for drug action, selectivity and resistance, antibiotic inhibitors of peptidoglycan synthesis, drugs and compounds altering the function of the cytoplasmic membrane, inhibitors of nucleic acid synthesis, inhibitors of the ribosome function and bacterial resistance to antibiotics. The book ends with a discussion on some possible experimental approaches to the antibiotic mode of action (double blockage, modification of existing antibiotic nuclei, and the use of alkylating agents).

The emphasis is on basic research, but the authors usually dedicate most of their contributions to drugs and antibiotics important for clinical use as antibacterials. Perhaps this is the reason for the omission of antibiotics acting on respiration and oxidative phosphorylation such as antimycin A, piericidin A, usnic acid, oligomycin, rutimycin and aurovertin.

It is stated in the preface that each author has written individually the chapter in which he is specialized and this has led to some lack of uniformity. For example, when dealing with inhibitors of nucleic acid synthesis, agents interfering with nucleotide metabolism are briefly described besides those drugs inhibiting either the enzymic process of nucleic acid synthesis or the template function of DNA. On the other hand, inhibitors of aminoacyl-tRNA formation such as borrelidin and furanomycin, and inhibitors of formyl-methionyl-tRNA_F formation such as aminopterin and trimethoprim, are not mentioned in chapter 6. In most cases, however, uniformity of style has been successfully achieved. All the contributors are, or have been for many years, members of the same sub-department of chemical microbiology in Cambridge working under the same head and it is very obvious the influence of this stage of their career in their style.

The book is well written and very carefully edited. The lists of references in each chapter are well selected and the field is well covered until August 1971; even many data not yet published at that time are included. The book is very pleasant to read and should be very useful not only to specialists on the increasing fields of antibiotic mode of action, but also to workers involved in teaching or those wishing to use correctly antibiotics as tools in research.

D. Vázquez

Oceanography

Seventy-Years Agrowing: a History of the International Council of Exploration of the Sea. By A. E. J. Went. Pp. 252. (International Council for the Exploration of the Sea: Charlottenlund Slot, Denmark.)

The International Council for the Exploration of the Sea was set up by eight European countries. Eight more, and Canada, joined later. There had already been national and international agreement to ensure good order among fishermen, but the great expansion of the industry following the introduction of steam propulsion, development of the otter trawl, and extension of markets by rail transport had brought fears of overfishing. Initiative for systematic study, coming from Scandinavia, was supported in the UK by prominent scientists, by the National Sea Fisheries Protection Association and by the Sixth International Geographical Congress meeting in London. Otto Pettersson, one of the most dedicated marine researchers of the day, helped by Nansen, persuaded the Swedish Government to call a planning conference in 1899 and the Council met for the first time in 1902. It has met annually for 70 years with some interruption during the two world wars.

From 1902 to 1908 it had its own Central Laboratory which proved a veritable cradle for oceanography, setting the pattern for half a century. The outlook then narrowed a little. While exploration added continually to our knowledge of the plankton and fishes of the Atlantic Ocean, the North Sea and the Baltic Sea, immediate problems such as mesh regulation, decline of herring stocks, dependence of recruitment on parent stock and building up reliable statistics were the main purpose. Progress was necessarily slow, but the Council has often been able to give advice of permanent value to the industry and has been the parent of a number of valuable conventions and commissions.

Dr Went deals with the scientific work and its achievements, but mostly in the context of organization, committees and personalities. He describes the early meetings as "rather family affairs with a few outsiders" and one sometimes wonders if the circle has been too restricted. It could, for example, have taken a more active part in the rapid widening of interest in marine science after the Second World War. It has since organized IGY work in "the ICES area", and arranged a symposium on physical variability, bringing an unusually wide range of scientists to one of its meetings.

It is clear that the Council has had excellent service from the small administrative and technical staff working at its headquarters in Copenhagen.

G. E. R. Deacon

Regulation in Cells

Control Mechanisms and Protein Synthesis. By S. D. Wainwright. Pp. vii + 550. (Columbia University: New York and London, November 1972.) £9.40.

The regulation of protein synthesis has long been one of the exalted topics in biology. Twelve years after its publication, the operon hypothesis has become firmly established as the primary mechanism for the negative control of gene expression in many bacterial systems. In spite of the obvious feasibility of the operon model as a general regulatory concept, it remains obscure to what extent this mechanism is employed in the more complex organisms. Incisive information has proved difficult to obtain because of the dearth of eukaryotic systems suitable for both genetic and biochemical analysis of the necessary detail. It is apparent, however, that eukaryotes have various control mechanisms which have no counterpart in prokaryotic systems.

Dr Wainwright's aim in *Control Mechanisms and Protein Synthesis* is to describe and assess the current state of our knowledge in this complex field. His view of the subject is broad, ranging from RNA-containing bacteriophages with only three genes to hormonal effects in metazoans. The treatment is detailed, though a more chemical approach might have been appreciated in those few cases where the molecular interactions are well enough understood. In places I found the sequence of chapters surprising. Thus the introductory chapter, "The System Regulated", a detailed and comprehensive account of the mechanism of translation, is followed by a contrastingly superficial chapter on differentiation and gene amplification. The synthesis of messenger RNA, often itself "the system regulated", is saved until chapters 5 and 6. Later chapters deal with molecular differentiation and antibody formation, devoting considerable space to models and hypotheses. A final chapter on recent developments ensures that the coverage is very up to date.

Dr Wainwright presents a balanced, if unexciting, account of the regulation of protein synthesis from the biochemist's viewpoint. The real strength of his book is its extraordinarily comprehensive bibliography which, together with the excellent index, will make it an invaluable guide to an increasingly daunting literature.

W. J. Brammar

INSTRUMENT REVIEW

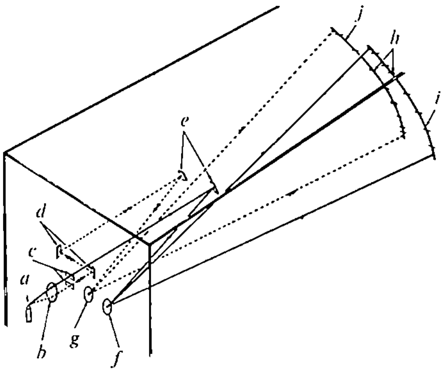
Analysis for Production Control

Polyvac E1000 System (Rank Precision Industries Ltd, from about £25,000).

PRODUCTION control of iron and steel, and of other metals and alloys, requires rapid and precise analysis of the molten base materials. Particular care must be taken that the composition of the sample does not change during the solidifying process, and that the time between sampling and analysis is as short as possible. Classically, analyses were made by wet chemistry, that is, the sample was dissolved, the element of interest separated and its concentration determined. Since the Second World War, physical methods of analysis, which are more rapid and do not involve highly trained staff, have increasingly displaced wet chemistry from the industrial routine analytical laboratory. The most common are spectroscopic techniques, in which the atoms of the sample are electrically excited to emit radiation, which is separated into its components by prisms, gratings or by other means, depending on the kind of radiation emitted (visible, ultraviolet or X-ray). The intensity of emission of a particular wavelength can then be converted into concentrations per cent by weight of the emitting element.

In the field of emission spectroscopy, a new vacuum direct reading instrument has recently been developed. This analysis system, consisting of the excitation unit, the spectrometer, a computer and a complete software package, makes it possible to carry out complete analyses of twenty-five elements within one minute. The first Polyvac E1000 system was installed at the Stainless Steel Works of Gebr. Böhler and Co. AG at Düsseldorf, Germany, and has been in continuous operation for the past year.

The excitation unit FS1630 used in the Polyvac E1000 system makes short excitation times possible by its feature of a variable discharge frequency. Repetition rates of 50, 100, 200, and 400 s^{-1} can be selected, resulting in a shortening of the sparking time from approximately 25 s at 50 Hz to about 3 s at 400 Hz, with the same efficiency of light production. Another important improvement in the FS1630 excitation unit is that individual discharges can be counted, so that the sparking is terminated when a preselected number of discharges have occurred. Independent of the internal standard (which is usually a matrix similar to the sample examined), the simultaneous analysis of all given elements of a sample has thus become possible at constant analysis times. In the past, analyses were made either against the internal standard, using variable analysis times, or against different standards using constant analysis times; normalizing to 100 per cent was indirect and involved calculations.



The optical path of the Polyvac E1000. *a*, Light source; *b*, condenser lens; *c*, entrance slits; *d*, periscope mirrors; *e*, plane mirrors; *f*, grating A; *g*, grating B; *h*, exit slits; *i*, focal curve A; *j*, focal curve B.

The second step towards reducing analysis time was made by integrating a digital computer into the system because, in the case of complex alloys, extensive calculations are required. The signals from the secondary electron multipliers are converted into concentration values (per cent by weight). Because a complex material omits a large number of spectral lines, some interference from neighbouring lines occurs, in spite of exact positioning of the exit slits. Clearly, this "noise level" depends on the composition of the sample and may vary considerably. It can, however, be corrected for, although a detailed discussion of inter-element and matrix effects would be beyond the scope of this review. To obtain an accurate analysis, all interfering factors must be recognized, calculated and corrected for. Only then can the first normalization to 100 per cent be made, a process which the computer can carry out within a fraction of a second of sparking.

The Digital Equipment computer PDP8/e, which is an integral part of the Polyvac E1000 system, not only performs these calculations but also controls the analytical procedure and monitors the maintenance of the given instrumental parameters. It requires a well developed software package; that used in the Polyvac E1000 system has performed very well since its installation. Some uncertainty was felt originally about replacing the conventional electronic controls by a computer. In retrospect, after a year of round-the-clock operation of the system, it can be said that the decision taken was the right one—the computer did not fail once during that entire period. Control and monitoring functions cover the control of electrical parameters, the argon flow, pre-spark and spark periods, and recalibration. The recalibration process is also fully automatic; it guarantees the analytical reproducibility of the method and is an important part of the system.

An essential component for accurate analyses is the spectrometer. The Figure shows schematically the optical path of this advanced apparatus, which offers the advantages of twin entrance slits, diffraction gratings, and exit slit plates. Thus the most intense spectral line for any element can be selected. For very complex materials with complex spectra it is obviously advantageous to have a combination of two suitable gratings, making it possible to analyse simultaneously spectral lines within the wavelength range from 1,596 Å to 8,640 Å or, depending on the problem on hand, to utilize the maximum reciprocal dispersion over the whole range.

The exit slits are engraved in the slit plate; by aligning the plate for one line, all other lines are aligned automatically, thereby saving substantial setting-up time. When the analytical programme is changed, it is fairly easy to replace one slit plate by another with a different exit slit arrangement. Each slit plate may have up to thirty engraved slits, corresponding to the maximum number of photomultipliers available.

The spectrometer can be equipped with either a single or a dual spark stand of the argon-flushed or air type. One advantage of a dual spark stand, where the individual spark stand is pneumatically brought into the proper position with respect to the optical axis of the spectrometer, is that samples of different shapes may be used. Another advantage becomes evident in routine work: if the throughput of sample is very high, a single spark stand may be heated by the thermal energy released during sparking, and this could introduce analytical errors. Changing from one stand to the other, which requires only 1 to 2 s, eliminates this source of error. Every sample is pneumatically clamped on the spark stand, so the spark gap width is very reproducible and rapid sample handling is possible.

The sensitivity of the photomultipliers can be adjusted very simply. Coarse step printed circuit boards make it possible either to select one of ten sensitivities by relocating a wire or to choose new sensitivities for each of the five analytical programmes, thereby increasing the versatility of the system. By means of the computer, five fixed analytical programmes can be stored in the system. These, in turn, can be divided into any number of subprogrammes by means of punched paper tape. Thus, even in case of complex high-alloyed materials, examination becomes possible.

Manual operation of this system is extremely simple. Introduction of the sample, selection of the programme and actuation of the starter button are the only operating steps which are carried out by the operator; calculations and printout of the results in per cent by weight are carried out automatically. Even for complex, high-alloy materials results are within the limit of conventional standard deviations—but are obtained within about 1 s.

Wolfgang Thomich

CORRESPONDENCE

Dingle's Answer

Sir,—While I accept and appreciate the apology on page 315 of this issue for the charge of dishonesty made in Professor Ziman's review[1] of my book, *Science at the Crossroads,* the circumstances are such that a further statement from me is called for so that the matter shall be rightly understood. It would, of course, be possible for me to have made false statements quite honestly, and readers of *Nature* might well infer, in the absence of comment from me, that such was the fact that had merely been expressed in the review by the wrong word. It is particularly necessary for me to remove that impression because the description of the book given by Professor Ziman, which has already been independently described in *Nature* as "admirable"[2], fails to make clear that its whole significance depends on *the truth of its factual statements.* "My purpose throughout", I wrote (page 19), "is not to indict but to inform, and let the facts bring whatever indictment is necessary." I cannot, therefore, justifiably allow any doubt to exist of the trustworthiness of those facts, for all of which I possess conclusive evidence.

This clarification is the more necessary because Ziman's opening sentence ("This is Professor Dingle's account of his attempt to persuade the scientific community that the theory of relativity is wrong and should be repudiated") must almost certainly suggest that to give such an account was the main, if not the only, purpose of the book. This would be quite false. I certainly should not have written it as a piece of autobiography, or even to try again to persuade the scientific community of anything. I wrote it, with great reluctance, only after 13 years of continuous effort in that direction had convinced me that further effort was useless, and it was because the *reason* for my failure seemed to me so ominous, both for the future of science and for the effect of scientific research on public welfare generally, that I had a moral obligation to make the whole facts known, free from my interpretation of them, and to leave readers to form their own judgment of what they implied. I referred in the preface (page 9) to my inability to obtain "the one essential desideratum of the whole exercise—plain evidence, through an answer to, or acceptance of, a very simple refutation of the immeasurably important special relativity theory, that the obligation to preserve strict integrity in science continues to be honoured", and I began the summing-up at the end (page 219) by saying "The primary and inescapable purpose of this book, which Part One attempts to fulfil, is to make known, to those with an indefeasible right to the knowledge, the present state of the scientific world as revealed by its practice, and to bring it into comparison with what is generally believed, and implicitly trusted, to be its state as typically expressed by the late Sir Henry Dale. I leave the reader to judge the significance of the comparison for himself, and to estimate what the consequences are likely to be if the present degree of conformity continues." This purpose is continually stressed in the intervening pages.

To prevent further misunderstanding I must add that part 2 ("The Intellectual Issue") on which Professor Ziman does not comment, though of equal length to part 1 ("The Moral Issue"), is of relatively academic interest. It is my reading of the historical causes of the change of attitude of scientists to their work, and is, of course, open to free criticism which I have not the slightest desire to suppress. Part 1, being incontrovertibly factual, is open only to reflexion, leading to whatever action concerned readers may think it proper to take. Professor Ziman's purported answer to my scientific "Question", which is the source and not the substance of part 1, has already been dealt with[3].

Yours faithfully,

Herbert Dingle

Purley, Surrey

[1] Ziman, J., *Nature,* **241**, 143 (1973).

[2] Ellis, G. F. R., *Nature,* **242**, 143 (1973).

[3] Dingle, H., *Nature,* **242**, 423 (1973).

Research Funding

Sir,—While it may appear fruitless to object to yet another cutback in federal funding for education and science, I do wish to protest very strongly about the recent reduction in a little noticed but extremely important programme—the National Science Foundation Undergraduate Research Participation programme.

Under grants from this programme, undergraduate science students conduct independent research projects during the summer months. The most gifted and dedicated students are exposed to the literature and techniques of the field, and are given the opportunity to design and execute their own projects under the aegis of an experienced professor.

This will be the fourth summer that I have supervised such a programme in the Biology Department at Massachusetts Institute of Technology. It is noteworthy that all of the students in this programme have gone on either to graduate school in the biological sciences or to medical school. Many of these students, while still undergraduates, published papers on their research or presented papers at national meetings.

My own interest in scientific research was kindled by four summers' research while in college and high school. My colleague Dr David Baltimore, a leader in cancer research and the study of

RNA tumour viruses, was first introduced to scientific research in NSF programmes. I could cite numerous similar examples from among the current leaders in virtually every field of scientific research.

This programme has been threatened several times with extinction. Funds for it were cut this year from $3.9 million in 1972 (supporting 2,600 students) to $2.0 million (supporting 1,300 students). The seriousness of this cut can be seen from the fact that, in all fields of the biological and medical sciences, and in all the colleges and universities in the nation, there will be space for only 308 students this year. Our own programme has been cut from 20 to 15 students. For these places we received over 160 applications and we were forced for lack of funds to reject many straight A and other very qualified students.

The scientific leaders of the USA during the next two decades are the gifted undergraduates of today; it is these students who are now being discouraged from entering research careers in all fields of science because of reductions of federal support in training and research funds at all levels. The NSF–URP programme, in particular, plays a central role in attracting gifted people into research careers, and its demise may have profound effects on the production of future scientific leaders.

Yours faithfully,

Harvey F. Lodish

Department of Biology,
Massachusetts Institute of Technology,
77 Massachusetts Avenue,
Cambridge, Massachusetts 02139

Nobel *Realpolitik*

Sir,—No doubt Eugene Garfield (*Nature*, **242**, 485; 1973) is correct about the correlation of citation frequency and Nobel Prizes. Such is the *Realpolitik* of science. But this indicates little about the true scientific value of frequently cited articles, and almost nothing about the value of those less frequently cited.

The *SCI* is a splendid bibliographic tool and may be a sociological tool as well. But I trust that the "rationale for thousands of infrequently cited journals" will not in fact be questioned merely on Dr Garfield's evidence. He should recall that Mendel's great paper was buried in an obscure journal, and Sir William Bragg's first seminal works rattled around ineffectually for a time in then-unknown Australian periodicals. There are quite a few other examples.

Thanks to *SCI's Permuterm Subject Index* such oversights need not now happen, but equally thanks to many infrequently-cited journals more really very good (and some really very bad) material gets into print. There is always the chance of another Mendel.

Yours faithfully,

James Friday

The Royal Institution,
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London W1X 4BS

Unanswered Questions

Sir,—One of your correspondents[1] asks those who, like myself, have referred to "flaws" and "unanswered questions" in evolutionary thought if they can supply examples. We certainly can—many of them. For space reasons I will quote only three.

Only four years ago a Darwinist grudgingly admitted that "Though it is nearly a century (*sic*) since Darwin wrote his treatise *On the Origin of Species*, there are still a few weak points in the theory of evolution. Often evolution seems to have made huge jumps, leaving no traces of any intervening steps and no hint that anything but the complete system could have functioned at all"[2].

It is surely incompatible with the scientific method to pretend that evolution is a proven fact, with these "huge jumps" unexplained. How did the whale evolve a complex nipple that enables it to suckle its young under water? This complex organ would have been worse than useless until it was fully developed, or Whale Junior would have received a mouthful of seawater instead of milk.

How did the giraffe evolve a long neck without first evolving the complex hydraulic control system that maintains an acceptable blood pressure in his brain whilst he lowers and raises his head? Or did he evolve his unique mechanism for regulating cerebral blood pressure before he evolved his long neck—and if so, how could such motiveless evolution have occurred?

Anadromous fish, grey whales and some species of migrating birds share an ability to find their way to an exact spot thousands of miles away. It is doubtful whether a submarine could equal their performance without the aid of shore-based radio beacons, even using today's highly sophisticated computer-based navigational systems. How did creatures so widely separated in the evolutionary tree as birds, fishes and mammals, all evolve these extraordinary navigational mechanisms? The problem is compounded by the experimental evidence that the spatial distribution of various elements in the oceans, the Earth's magnetic field, and the position of the stars all play a part in the overall phenomenon. Yet over geologic time these three parameters have been varying continuously; how, then, did these creatures evolve the ability to navigate against a moving datum?

If evolutionists would face these questions—and hundreds of others like them—honestly, they might both advance their branch of science and gain a little more respect for those Bible-believing scientists who see no incompatibility between science and their Christian faith.

Finally, let me point out that your correspondent is a couple of generations out of date in his glib assertion that "any archaeologist, theologist or philosopher could tell them that many of the stories in the Bible are copied from the folk-tales of long-ago tribes more ancient than the Hebrews".

As I have shown at some length[3] there are two schools of thought even amongst theologians about the accuracy of the Old Testament's historical narratives—and a considerable proportion of the world's leading archaeologists now regard these narratives as highly accurate records that are independent of, and in many cases precede, the legends of other ancient peoples.

Yours faithfully,

A. T. J. Hayward

2 Wellington,
East Kilbride,
Glasgow G75 8RB

[1] Angseeing, J. P. A., *Nature*, **242**, 214 (1973).

[2] Anonymous, *New Scientist*, December 11, 542 (1969).

[3] Hayward, A., *God's Truth: A Scientist Shows Why it Makes Sense to Believe the Bible* (Marshall Morgan and Scott, London, 1973).

Errata

The title to the article by M. Koltai and E. Mecs (*Nature*, **242**, 525; 1973) should read "Inhibition of the Acute Inflammatory Response by Interferon Inducers", not ". . . Interferon Inhibitors".

In the article "Sexual Behaviour and Sexual Motivation in the Female Rat" by R. F. Drewett (*Nature*, **242**, 476; 1973) the first sentence in the last paragraph should read "Oestrus is associated with enhanced general activity but this seems to be independent of sexual receptivity".

In the article "Sativin: an Induced Isoflavan from the Leaves of *Medicago sativa* L." by J. L. Ingham and R. L. Millar (*Nature*, **242**, 125; 1973) paragraph 11, line 14, should read "sativin" instead of "sativan" and paragraph 12, line 6, should read "lonchocarpan" instead of "lonchocarpin". The contents page entry should read "isoflavan" instead of "isoflavin" in both cases.

In the article "Further Data on the Corsica–Sardinia Rotation" by F. Radicati di Brozolo and G. Giglia (*Nature*, **241**, 389; 1973) paragraph 23, line 11, "lower" should read "upper" and "upper" should read "lower".

Corrigendum

IN the article "Cyclical Changes in the Habitat and Climate of an East African Ecosystem" by D. Western and C. van Praet (*Nature*, **241**, 104; 1973) in Fig. 3, $r = -0.92$ and the equation should read, $\log y + 1 = 2.87 - \log x$.

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Economics, Animals and Pollution

PROFESSOR HARRY JOHNSON, economist, American, holder of chairs at the London School of Economics and the University of Chicago, and a former member of the now defunct Council for Scientific Policy, has come down hard on the so-called environmentalists. In a well written and instructive booklet prepared for the benefit of the British–North American Committee, Professor Johnson brings the power of economic reasoning and, it might be said, common sense to the problems of man and his environment, and his arguments deserve to be read by all who profess to have at least a passing interest in these problems (*Man and His Environment,* British–North American Committee, £0.40).

It seems that there are still people who need convincing, or even teaching, that economic growth produces something other than pollution. Those who advocate bringing the economy to a standstill so that further damage to the environment can be avoided do so, it seems, without fully realizing the implications. Quite often it is these very same people who, quite properly, call for all efforts to be made to improve the lot of people living in the developing countries. But how can this be achieved without economic growth? The advantage of having professional economists turning their attention to these problems is that the most perceptive of their profession soon come up with the answer that growth and preservation of the environment are not necessarily incompatible.

The arguments for this are by now well publicized and Professor Johnson reiterates them to effect: "Economic growth and not the impediment of it is the way to a betterment of the quality of life and the avoidance of ecological disaster." He argues, quite cogently, that what are often considered the chief environmental problems of today—air and water pollution, deforestation, apparent shortage of mineral resources—are not the most important ones. Air pollution can be decreased by attacking its source, either by technical developments which are already available, or by continuing research into, for example, making the internal combustion engine more efficient and therefore less of an environmental hazard. Water pollution can similarly be tackled while forests can be replanted and minerals recycled. This is not to say that these programmes of work will present no problems, but there is no evidence to show that the ingenuity of man will not, as it has so often done in the past, provide a solution.

There are, however, other problems to which the environmentalists have attached their flag. And it is these which are more deserving of attention. Wild animals and fish once they are hunted to extinction are a permanent loss and no amount of technology (or genetic engineering for that matter) will bring back extinct species. It is in this field that there is need for the greatest concern. But in some cases, clearly, a compromise will have to be reached where the animal provides a living for some people or groups of people and where a wholesale ban of hunting would not be totally beneficial.

A case in point is the concern now being expressed once again for the survival of whales worldwide. There are those who would ban whaling for several years to ensure that some species which are most threatened will increase their stock without danger. But is there a need to jeopardize the livelihood of so many fishermen and others who make their living out of whale products in order to preserve these aquatic mammals?

Would not a more sensible approach be to try and extend the ban already in existence on some whale species to others which are in danger? To ensure the success of such an approach all the whaling nations, the largest of which are Japan and the Soviet Union, must cooperate to the full, and even though such an approach might be difficult to police, it will stand a much better chance of being approved by the International Whaling Commission which meets in London at the end of the month than a blanket ban on whaling.

A far more worthwhile campaign than attempting to have a moratorium on whaling would be to persuade the whaling nations which are not members of the commission to either become members or observe the quotas and bans imposed by the commission.

Professor Johnson, quite properly, is more concerned with the conservation of animals than pollution, at least in so far as he sees solutions to the latter problems, but his faith in science which is evident in most of his booklet deserts him when it comes to problems which cannot transparently be subjected to economic analysis. "It is not established," he says, "that we have the scientific capacity to remedy the damage we may be doing to our oceans and upper atmosphere by treating both as a costless medium for the transport of people and goods and the oceans as an inexhaustible source of fish and crustaceans for human nourishment." Where, Professor Johnson, have you lost your faith in the ingenuity of man?

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100 Years Ago

A PROJECT has been set on foot by Colonel Grant, so well known from his African travels, to form a loan exhibition of skulls and horns of hollow-horned animals, in order that by observation and comparison of a large number of characteristic specimens, facts may be obtained regarding the form, sexual characters, and locality of each particular species. It is proposed to have as many as from twenty to fifty specimens of each species, so as to be able to form groups representing every stage in the life of each, as also to show the varieties of species in different localities. When from three to five thousand specimens of the one hundred and fifty existing species has been promised, means will be taken to secure the most suitable place in London for their exhibition.

From Nature **8**, 151, *June* 19, 1873

OLD WORLD

Forging Links Between Steelmaking and Nuclear Power

THE linking together of steelmaking and nuclear power is now a sufficiently real prospect, albeit ten years or more away, for representatives of European steel and nuclear companies to have decided in principle to form a club to look into the possibilities and problems more fully. This move was made at a discussion meeting, convened last week in London by the British Steel Corporation and the British Nuclear Forum, at which it was announced that Japan is to spend about £10 million on research into nuclear steelmaking during the next six years.

Although the meeting left the details of the club—who should be eligible for membership, how much money should be spent and precisely what the objectives should be—to be decided in future consultations, it did provide a useful opportunity for airing the differences between what the steelmakers require of a nuclear power plant and what the nuclear people think they can offer.

One of the basic reasons for the steel industry regarding nuclear power as an attractive source of energy is that the coking-coal now used to produce the coke for blast furnaces is likely to become considerably more costly and scarce in the future. And of the longer term alternatives—such as replacing some of the coke in a blast furnace by oil or natural gas or converting other coals to coke—the most attractive seems to be direct reduction of ore by a hydrogen and carbon monoxide gas.

The so-called reforming stage, at which hydrocarbons are converted into suitable reducing gases, requires heat at 900° C or so and this is where a nuclear plant in the form of a High Temperature Reactor (HTR) would come in. Helium coolant at a temperature of about 1,000° C could be used as the transfer medium and reducing gases could be produced at a temperature of about 800° C. This temperature is critical because if the hydrogen is any colder the reduction of ore pellets in the favoured "continuous shaft process" can take excessive times and reduction of the pellets may even then not be complete because of reoxidation. (On the other hand, at higher temperatures the ore pellets have to be carefully selected because of the problem of "sticking".)

What do those concerned with HTRs have to say about the problems? Dr L. Shepherd, head of the OECD Dragon HTR project, said that helium temperatures of 1,000° C can now be produced for long periods at Dragon with little difficulty and that it should ultimately be possible to raise this by perhaps a few hundred degrees. He went on to say that the design of heat exchangers capable of operating at the temperatures and pressures required seemed to be the most difficult problem. Ceramic exchangers—made, for example, of silicon carbide or silicon nitride—would be lighter and cheaper than metal ones but their performance at high pressures may prove to be a stumbling block.

Another factor that has to be taken into account is that an integrated nuclear steelworks would have to be near a populated area and therefore safety would be a paramount consideration. Because of this, most of the discussions at the meeting assumed that there would be a secondary helium circuit to ensure that radioactive contamination would be minimal. Fortunately the HTR is considered by many to be the safest available reactor type. There is another possibility, however ; reducing gases could be produced with reforming plant at a remote nuclear site and then pumped cold to a steelworks, where they could be reheated by conventional means or by self-burning.

Nature Subscriptions

THE *Nature* subscription list has been computerized. Each address label now carries a reference consisting of three letters and four numbers. Any queries relating to subscriptions should in future quote this reference.

SELECT COMMITTEE

Science Adviser Likely

THE Select Committee on Science and Technology may be about to acquire a full-time science adviser. Mr Airey Neave, chairman of the committee, is understood to have approached Mr James Prior, leader of the House of Commons, asking for his comments on various options put forward by members of the committee.

Although the committee has not discussed the proposal formally as yet, there has been pressure from a number of members for a full-time secretariat. The proposal—if it is accepted—would move the select committee a little nearer the Congressional committee system in the United States where the powerful House committees often have highly qualified secretariats which write and commission background reports for the committees' members.

If Mr Prior approves the scheme, the science and technology committee will be the first of the select committees to appoint a full-time adviser. Such an appointment would not, however, replace the *ad hoc* appointments that the committee makes from time to time—for instance it is advised on computer matters by Professor A. Douglas of the London School of Economics.

As the committee has yet to formally consider the proposal and as Mr Prior has not yet replied to Mr Neave, the precise role of the adviser has still to be defined. It is unlikely, however, that he would be an academic scientist. Rather the select committee is likely to look for someone who can find out government attitudes on various matters for the committee.

In line with the select committee's desire to ensure that Mr Heath's government is in fact "open government" it is likely that the correspondence over the appointment of the adviser will be published, if in fact approval for the appointment is given.

Meanwhile the committee is finalizing its reports on nuclear reactor policy and computers. Both will be published in about a month's time, as will the committee's report on the hovertrain. When that appears it is more than likely that Mr Michael Heseltine or, conceivably, Mr Peter Walker, will be invited by the committee to comment on its findings. For a vehicle that no longer runs the hovertrain seems to be an issue with plenty of mileage left in it.

The committee also hopes to finalize its plans to visit Brussels shortly. The visit, originally planned for May, will now take place in the week beginning July 9. It is probable that the committee will take evidence from Commissioners Dahrendorf and Spinelli, as well as from Mr Christopher Layton, Director for Advanced Technology Industries at the EEC. It is also likely that the select committee will see the commission's directors on energy and nuclear power, and a day visit to Ispra, one of the laboratories of the Joint Research Centre, may be included in the itinerary.

ANIMAL BREEDING

Bullseye

THE first deep-frozen bull has been born. Late last week a healthy bull calf was born to a cross bred Hereford-Friesian cow at the Agricultural Research Council's Unit of Reproductive Physiology and Biochemistry at Cambridge. Nine months ago the embryo of the calf was immersed in liquid nitrogen having been removed, after fertilization, from the uterus of its true mother.

The bull calf is the first large mammal to be born after being deep-frozen as an embryo. If the technique is perfected it could have large commercial implications for the livestock industry.

In all, the Cambridge unit transplanted twenty-two eggs into eleven cows some nine months ago. The eggs all came from normal mothers fertilized by artificial insemination, and were removed from the uterus on day 10 of pregnancy, in the blastocyst stage.

The eggs were then frozen to −196° C with dimethylsulphoxide, a cryoprotective (antifreeze) agent, replacing some of the water in the embryo cells to prevent ice forming within them. Having been cooled very slowly at 0.2° C a minute they were kept frozen for six days. They were then thawed at a rate of 360° C a minute in a 37° C water bath, and transplanted into the uterus of the host mother.

Only two of the eggs became implanted in the host uteri. Six weeks into pregnancy one cow was developing two embryos, but one of these died later in the pregnancy. Nonetheless, one bull was born successfully.

This success with cattle follows the development of the freezing technique with mice in the summer of last year by the Cambridge unit and by the Oak Ridge National Laboratory in the United States. The mouse and cattle programmes were in fact running virtually in tandem, but the faster rate of reproduction in mice naturally brought success there before success with cattle, which have a gestation period of about nine months. Similar experiments with pigs and sheep have not as yet been successful.

The failure with ten of the cows is attributed to damage to the embryos during freezing and thawing.

Dr Wilmut, one of the research leaders at the Cambridge unit, said this week that the freezing and thawing process undoubtedly kills some of the embryo cells. Because some differentiation has occurred in the blastocyst stage it is probable that in the unsuccessful attempts a number of vital cells were killed, whereas in the successful attempt the cells killed were replaceable.

Experiments already carried out with earlier stages of embryo development have not been successful, possibly because of the presence of the zona pellucida, a relatively tough membrane which surrounds the ovum and which complicates the freezing process.

Future experiments, Dr Wilmut said, will involve varying four factors—the stage of development at which the embryo is removed from its true mother, the rate at which the embryo is frozen, the rate at which it is thawed, and the composition of the medium in which it is frozen. Any one of these factors is crucial.

The potential value of the technique, however, makes careful experiments that may lead to a commercial system well worth while.

If perfected commercially, milk cows could be used to breed beef cattle. The saving would lie in not having to keep beef cows alive purely for breeding purposes. Milk cows could still be used for milk production before and after they carry beef cattle.

Equally the technique would allow genetic characteristics that are in danger of being bred out of cattle for economic reasons to be kept in cold storage for the day when these characteristics may again become commercially important. At present if certain gene strains are bred out then there is no way they can be recovered.

Entire herds could be transported around the world in liquid nitrogen flasks, and countries such as Australia, which are still free of certain European diseases, could import frozen embryos that time would have shown to be disease free, whereas at present cattle imports are strictly limited.

The technique would also allow for a careful record of genetic change to be kept, as frozen embryos could be developed decades after the eggs were first fertilized. The resulting animal could be compared to the types that have developed by natural selection. This could in time improve selection methods in cattle breeding.

But all this may be many years away. Developing a commercial freezing method with a high success rate could be a long job, and various refinements to the peripheries of the technique will be needed before frozen embryos become comercially available. For example a non-surgical method of transplanting the embryos will have to be devised as surgery is time consuming and expensive. But these problems are likely to prove quite minor. The problem that really needs solving is how to guarantee a high success rate.

SOVIET SCIENCE

Of Machines and Men

from our Soviet Correspondent

LUNOKHOD-2, which was rolled out on to the lunar surface on January 16, 1973, has completed its working programme after a life of five lunar "days" in which it traversed some 37 kilometres (approximately 3.5 times as far as the less mobile and less manoeuvrable Lunokhod-1).

Although in many respects the Lunokhod-2 programme continued its predecessor's work—spectrometer and penetrometer investigations of the chemical and physical properties of the surface rocks, the investigation of solar and galactic corpuscular radiation, and a continuation of the Franco-Soviet laser range-finding research to determine the exact distance from the Earth to the Moon (see *Nature*, **228**, 795 ; 1970)—certain features of the programme appear to be new.

The choice of landing site, on the eastern edge of Mare Serenitatis, enabled a detailed television study to be made of the transition region between mare and highland. In all, over 80,000 television pictures were received, with a stereoscopic system providing detailed interpretation of the relief—a matter of especial importance during the investigation of a major tectonic fault on the fourth working "day" (April 11–22, 1973).

Special emphasis, too, was given to the magnetic experiments. The greater range of mobility of Lunokhod-2 enabled variations in the magnetization of the surface rocks and variations of the lunar magnetic field to be measured over a fairly extensive track.

Another innovation was the use of laser direction-finding apparatus to determine the luminosity of the lunar sky in the visible and ultraviolet regions.

According to Academician Boris N. Petrov (*Pravda*, March 25, 1973), the Lunokhod programme of automatic lunar exploration, with its potentialities for long-term and long-range study of the surface, is envisaged as one of the main projects of the Soviet Space Programme, equal in importance with the Saylut series of orbiting space stations. Academician Petrov is closely connected with the planning of both types of research (he is one of the chief organizers of the proposed Soyuz/Apollo flight, and is chairman of the IFAC Committee on Automatic Control in Space) and may therefore be assumed to speak with detailed knowledge of both branches. In view of the recent setback to the Saylut programme, however (see *Nature*, **243**, 181 ; 1973), and the undoubted success of Lunokhod-2, a rethinking of priorities in Soviet space planning may well be in progress, with even greater emphasis being given to automata.

NEW WORLD

Change of Tack on the Shuttle

by our Washington Correspondent

THE long and convoluted debate about the space shuttle has taken a strange and confusing turn. A Congressional agency has taken a damaging swipe at NASA's economic justification for the project, but NASA officials, undaunted, have come up with a fresh analysis claiming that the shuttle is an even better economic bet than they had previously thought. In Congress, meanwhile, critics of the shuttle are arming themselves with a new set of arguments to use when NASA's budget comes up for debate in the Senate, but even the project's staunchest opponents are privately conceding that they have little chance of choking off the funds this year, at least.

What it all adds up to is more confusion in an already confused situation. By now, the shuttle is perhaps the most intensely studied technological project to come before Congress, but as an example of prudent science policy making, the whole debate leaves a lot to be desired, chiefly because it has been concerned almost exclusively with the economics of the project while its wider implications have been underplayed. The real issue is the shape and balance of the US space programme for the rest of the century.

Congressional opponents of the shuttle, led by Senator William Proxmire and Senator Walter Mondale, last year set the General Accounting Office the task of examining the basis of NASA's claims that development and use of the shuttle will save the agency money when compared with the cost of launching satellites with conventional, expendable rockets. The GAO has some harsh things to say about NASA's figures, but its report, published last week, is remarkable chiefly for the fact that it reveals for the first time that NASA has recently come up with a completely new economic analysis of shuttle operations.

When the final shuttle design was chosen last year, NASA argued that although it would cost some $8,100 million to develop and produce, use of the shuttle would save some $5,200 million between 1978 and 1990. The analysis rested on the assumption that NASA's budget would be able to support 581 shuttle flights in that period, and that the chief savings would result from the fact that the shuttle will enable satellites to be repaired and refurbished after they had been placed in orbit, and from removal of size and weight constraints in payload development. But, on April 27—three days before the GAO originally intended to publish its report—NASA sent the accounting office a completely new mission model. Instead of 581 shuttle flights up to 1990, the agency is now suggesting that its budget will be able to support 779, and the estimated savings have shot up from $5,200 million to about $16,000 million.

GAO has not had time to examine these drastically modified estimates in detail, and the effect has been to torpedo, to some extent, its analysis of NASA's original estimates. The new mission model has neither been explained in detail by NASA nor subjected to independent analysis, but according to Mr Dale Myers, head of the Office of Manned Spaceflight, one of the chief differences from the original model is that it assumes that one third of the shuttle flights will be sortie missions, using the spacelab which will probably be developed by the European Space Research Organization (see *Nature*, **241**, 418 ; 1973).

The spacelab is a pressurized capsule in the shuttle payload bay, which will house up to six scientists, and an unpressurized unit that will be used for large instruments such as telescopes and antennae. The unit will remain attached to the shuttle throughout the mission and will be removed on landing and prepared for the next flight. Mr Myers said in a telephone interview last week that the spacelab is expected to reduce payload costs substantially, so that more

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AIR POLLUTION

Relief for Detroit

by our Washington Correspondent

THE Environmental Protection Agency last week published some data to back up its assertion that nitrogen dioxide does not present a widespread air pollution problem in the United States. In fact, the agency now reckons that only two cities have levels of nitrogen dioxide that pose a potential hazard to health, while a year ago 47 regions were accorded that distinction. Sighs of relief were clearly audible in Detroit at the EPA's reappraisal of the situation.

The reason for the rather dramatic change in the status of nitrogen dioxide is that the method that was originally used to measure concentrations of the pollutant in the air has recently been found to have given greatly elevated readings. The result is that only Los Angeles and Chicago are now reckoned to have levels of nitrogen dioxide greater than 100 μg^{-3}, the level above which it is considered to be harmful to health. There is also some question about New York and Salt Lake City, where the EPA is rechecking its measurements.

The importance of all this for the automobile manufacturers is that the Clean Air Act commands them to produce cars in 1976 which emit 90 per cent less oxides of nitrogen per mile than those produced in 1971. That requirement was based on the original faulty measurements, and the manufacturers are now claiming that such stringent controls are not necessary. In order to meet the requirements of the act, the car makers would have to fit a second catalyst to the conventional engine, in addition to the converter needed to remove hydrocarbons, and a committee of the National Academy of Sciences reported recently that there is considerable doubt about the reliability of the dual catalyst system.

The EPA's new analysis suggests that even if no stricter automobile emission controls than those now in operation are applied, only Baltimore, in addition to Los Angeles and Chicago, will have levels of nitrogen dioxide in 1985 that exceed the 100 μg^{-8} level. The EPA is allowing 45 days for comments on its new analysis, after which it will turn all the data over to Congress with a suggestion that the requirement for 90 per cent reduction in exhaust emissions be modified. The chances are that Congress will probably end up modifying the Clean Air Act so that car makers will still have to reduce emissions of oxides of nitrogen substantially, but not to the extent of fitting dual catalyst systems to car exhausts.

In the meantime, however, the EPA must hold public hearings and decide whether or not Chrysler should have an extra year to meet the present standards prescribed by the act. As with hydrocarbon emissions standards (which are not affected by the reappraisal of oxides of nitrogen) the EPA Administrator can grant manufacturers an extra year to comply if he finds that the technology for meeting with standards is not available, and late last month Chrysler requested such an extension. The EPA's decision must be made by August 29.

flights can be fitted into NASA's budget. He also pointed out that the lab will be a unique capability of the shuttle, and thus direct cost comparisons with missions using conventional launchers are difficult to make. One possible extension of NASA's reasoning is that if the shuttle is used to carry out many sortie missions, there will be less justification for building a space station, which will also free more money in the 1980s for other missions.

Although NASA's new estimates have made the GAO's investigation of the economic arguments for the shuttle redundant in some respects, the accounting office's report contains much information which will undoubtedly be used to good effect by the project's critics. In short, the GAO concludes that it "is not certain that the space shuttle is economically justified . . . even though NASA's calculations show that it is". The report has reservations about possible cost overruns in development of the shuttle, and, more important, uncertainties in the overall size of the space programme.

In particular, the GAO points out that if there is an austere space budget so that there are many fewer shuttle flights than planned, development costs of the shuttle would not be spread so thinly, and the economic balance may be tipped in favour of expendable launchers. The report notes in several places that uncertainties in shuttle costs suggest that the choice of launcher system "should not be based principally on cost estimates".

Only in one instance does the GAO come up with a figure that is radically different from NASA's analysis, however. NASA officials have suggested in testimony before Congressional committees that the shuttle will be able to put a pound of payload into orbit for only $160, compared with between $900 and $5,200 with expendable launchers. But the comparison is "not a meaningful one", the accounting office suggests, because NASA neglected to include the cost of development and production of the shuttle, and also computed the figure on the assumption that the shuttle would be used to maximum capacity. If corrections are made for those factors, the GAO suggests that the launch costs per pound are nearly $3,500.

The GAO report and NASA's new mission model are sure to generate considerable controversy, and cloud the issue even further. But there are signs that Congressional opponents of the shuttle are beginning to use more straightforward arguments that go to the heart of the project. During hearings earlier this year before the Senate Committee on Aeronautics and Space Science, and the Senate Appropriations subcommittee that deals with NASA's budget, Senator James Abourezk and Senator Proxmire have been asking some sticky questions about the short term effect of shuttle development on other items in NASA's budget.

This year, NASA is spending $200 million on shuttle development. Next year the figure is expected to rise to $475 million, and by 1977 it is expected to reach nearly $1,200 million. Critics of the project want to know what effect that growth will have on NASA's space science and applications programmes. The crux of the matter is whether the Office of Management and Budget will allow the agency's budget to recover from the decline which has set in during the past few years. NASA officials say that they are confident that they will soon get back to the $3,400 million level, but its critics say that there is no such guarantee.

The fact is that OMB allowed NASA only about $3,100 million for 1974, and the projections for 1975 which were published with the 1974 budget request allow for only a modest increase to $3,160 million. If the agency's budget stays at that level, critics of the shuttle have been suggesting that the science, technology and applications programmes will clearly suffer.

Whatever the force of those arguments, however, it is unlikely that the project will be stopped by Congress this year. Already the House of Representatives has easily turned back an attempt to delete funding from the shuttle from the NASA authorizations bill, and even the chief critics in the Senate are not optimistic about their chances. For one thing, they could only garner 21 votes last year against the shuttle, and that was the crucial vote which allowed NASA to go ahead and let contracts for the project. Another reason why the shuttle will be hard to stop is that the spectacular success of the improvised repairs to Skylab have at least demonstrated that man has some use in space. NASA will be sure to use that argument with telling effect in support of the manned space flight programme.

BIOMEDICAL RESEARCH

More Protests

NINE Nobel laureates, backed by the 6,000 members of the Federation of American Scientists and 2,000 other scientists last week sent a letter to President Nixon protesting at cutbacks in funding for biomedical research. The letter indicated two "fundamental complaints" about the Administration's proposed budget for 1974, namely the fact that increases in the budgets of the National Cancer Institute and the National Heart and Lung Institute are being offset by cutbacks elsewhere, and the decision to phase out NIH training grants and fellowships. Those complaints are by now familiar, for the biomedical community has kept up a barrage of protests since the Administration's budget was unveiled in January.

The letter does point out, however, that in the face of financial stringency in all parts of the federal budget, funding for biomedical research cannot be expected to increase rapidly in the next few years, but adds that "we have a right to expect that the funds which are available to our discipline are allocated in a sensible way".

VENUS

Still Planning to Go

by our Washington Correspondent

IN spite of two setbacks, NASA officials are pushing ahead with plans to study Venus. A team of investigators has been chosen to provide experiments for a Pioneer spacecraft which, if all goes well, will send four probes into the Venusian atmosphere in 1978, and plans are also being drawn up to send an orbiter to the planet in the same year. In addition, there is another launch window in 1980 which will probably be used to launch a second orbiter or, if the 1978 probe mission fails, a second set of probes.

Those plans are, however, less ambitious than the programme that was being talked about last year, when NASA was hoping to send two sets of probes to Venus in 1976, an orbiter in 1978 and another orbiter in 1980. The first to be dropped was one probe mission which went by the board in November during bargaining between NASA officials and the Office of Management and Budget over the agency's 1973–74 budget (see *Nature*, **240**, 177; 1972). But OMB officials still refused to allow NASA to put in for the money, and the 1976 launch has slipped to 1978. The second setback came earlier this year, when the European Space Research Organization decided not to join in the orbiter mission because of the uncertainties about US funding.

The 1978 probe mission will be launched in May, and arrive at the planet in December of that year. It will send one large probe, carrying about 60 pounds of instruments, and three small probes each carrying three pounds into the atmosphere. The large probe will take about 90 minutes and the small probes about 75 minutes to reach the surface, when their data transmission will stop. As for the orbiter mission, scientific experiments will be chosen in January 1974, and although ESRO has opted out of the mission, NASA officials are exploring the possibility that Britain or the Federal Republic of Germany will join in.

NEWS AND VIEWS

Vaccination Policy for Smallpox

FEAR of smallpox has again renewed controversy on the policy of routine vaccination against this infection in non-endemic areas such as the United Kingdom. Smallpox ceased to be endemic in this country in 1935 and all but two outbreaks, including the recent outbreak which presumably originated in a laboratory at the London School of Hygiene and Tropical Medicine, have arisen from importations. Indeed, since 1950 there have been 378 cases of smallpox in the United Kingdom resulting in 60 deaths. Alarming as these figures appear, the risks of vaccination complications may nonetheless outweigh the benefits of routine vaccination in non-endemic situations. Thus between 1951 and 1970 there were about 100 deaths from smallpox vaccination in England and Wales (Dick, *Brit. med. J.*, **3**, 163 ; 1971). Even so, these figures may be incomplete because there is probably some under-reporting, and non-fatal complications have been excluded. This is still perhaps not the best way to balance the policy of universal vaccination, the risk of smallpox and the complications of the vaccination procedure itself.

Herd immunity is still a common philosophy, and there is little doubt that a vaccinated community has an advantage, as far as mortality rate is concerned, over an unvaccinated community. It has been estimated, however, that in 1947, even after allowing for mass vaccination during the war and endemic variola minor during the period 1920 to 1934, the total immunity in England and Wales was less than 20% (Dixon, *Smallpox*, Churchill, London, 1962, p. 342). The acceptance rate for primary infant vaccination has been perhaps 40% and for revaccination probably less than 10%. In order to establish a high herd immunity it would be essential to have successful universal revaccination, every 3 years, throughout life. But even apart from the risks of vaccination it is difficult to justify immunization against a disease which has been successfully eradicated from all but seven countries—Bangladesh, Botswana, Ethiopia, India, Nepal, Pakistan and Sudan (*World Health*, October 1972). Because of the success of the eradication programme of the World Health Organization and the concomitant decline in the risk of smallpox importation, the United States and the United Kingdom discontinued in 1971 the practice of routine vaccination.

Severe outbreaks of smallpox from importations have been controlled for many years now by isolating cases, and tracing, vaccination and surveillance of known probable contacts. This policy of ring vaccination has been remarkably effective. There is, therefore, no place for mass unselective vaccination during a limited outbreak. Following the recent London incident some informed newspapers attempted to discourage mass vaccination, unlike the situation accompanying the outbreak of smallpox in South Wales in 1962, when at least eighteen people died from indiscriminate vaccination (Dick, *loc cit.*), generated to a large extent by panic.

At the same time it is imperative to vaccinate and revaccinate and to ensure successful vaccination of high-risk groups such as doctors, nurses, laboratory workers, ambulance drivers and other hospital and public health personnel. In Europe during the period 1950 to 1971, for example, smallpox killed, among the hospital staff, an alarmingly high proportion of its young adult victims, fourteen or 70% of the twenty without previous vaccination (Mack, *J. infect. Dis.*, **125**, 161 ; 1972). The policy of selective and ring vaccination must continue, therefore, at least for the time being.

From our Medical Virology Correspondent

Diamond and Cavitation

WHY are diamonds found naturally in geological circumstances which belie the high pressures needed to synthesize them? This is the question which Galimov considers on page 389 of this issue of *Nature*. He comes to the interesting conclusion that diamonds could form when cavitation bubbles in flowing kimberlite magma collapse. The point is that cavitation can occur when the pressure on a liquid with gas dissolved in it is reduced below some critical value. Cavitation can take place, for example, in the vicinity of propeller blades on ships and in those circumstances can cause considerable damage.

Kimberlite usually occurs in vertical pipes a few tens of metres in diameter and a few kilometres long. Galimov calculates that magma flowing at a pressure of 20 kbar or so could be induced to cavitate if the magma passed through a constriction the cross-section of which is only two to three times smaller than that of the neighbouring regions of pipe. If one applies the celebrated Bernoulli relation it turns out that the pressure in these circumstances is reduced below that required for complete dissolution of carbon dioxide at 1,000° C. Further calculations reveal that the pressures and temperatures reached locally when cavitation bubbles collapse would be such as to allow the synthesis of diamonds.

Galimov also deals with the question whether the extreme conditions that exist when a cavitation bubble collapses last for long enough for a diamond to form. Extrapolating from the conditions in which diamonds have been artificially created in explosions, he calculates that appropriate natural conditions probably exist for a few milliseconds at a time, three orders of magnitude longer than is the case in a typical explosion experiment.

The cavitation concept also explains why some diamonds seem to have grown in a series of discrete steps: a newly formed diamond would evidently be a good site for the formation of subsequent cavitation bubbles and during each collapse the diamond would increase in size. And because the high pressures only occur very locally, other minerals usually formed in such conditions are not found in kimberlite.

CARCINOGENESIS

Testing Time for Toxicologists

by our Special Correspondent

A CHALLENGE has been thrown out to toxicologists and cancer researchers by the growing demands that new and existing drugs should be tested for carcinogenicity. At present the resources of materials and techniques available to them are limited and they must quickly look for new and improved methods. Taking up the challenge, the Drug Research Board of the US National Academy of Sciences called a conference in Washington DC on May 23–25 to consider carcinogenesis testing in the development of new drugs.

The essence of the procedures most commonly used to test for carcinogenesis has been to treat animals—usually rats and mice—with the drug for varying times, to look for effects on the time of appearance and the number and types of tumours that develop. There are, however, many problems. For example, what strain of animal should be used; how much drug should it receive for how long; and what diet should it eat while being dosed? These, and all the other questions tackled at the meeting, were first thrashed out during day-long discussion groups, whose leaders reported their recommendations during the second and third days.

Dr M. B. Shimkin (University of California, San Diego) recommended, on behalf of his group, that inbred strains of mice provide the best single animal for testing, but that rats are also desirable for this purpose, with the Syrian hamster probably the next best species. Larger animals, including primates, however, are impractical for bioassays and should be restricted to special problems. Nevertheless, the use of more non-rodent species should be explored.

The group concerned with the size of doses recommended, through its leader Dr L. Friedman (Food and Drug Administration), the use of the maximum dose that permits valid interpretation of the experimental observations. Usually, the group decided, with rodents this dose should be such that it will not depress the increase in weight more than 10% compared with controls. This dose should also leave 50% of treated animals alive at the end of the study and produce no pharmacological effects to invalidate the results. Two other doses should be used, however, the second highest usually being 10–50% of the maximum. Dr Friedman's group recommended that treatment, which should begin at conception and continue until completion of the study, or death, should be given on five to seven days each week. The route of administration to the animals should be oral or topical when they apply to human use of the drug. Intramuscular and subcutaneous injection should be used only if absorption is poor in the test animal after oral administration, and inhalation should be limited because animal models are not sufficiently established to yield valid negative results with substances of unknown carcinogenic potency.

Diet can affect considerably the progress of carcinogenesis in rodents, and so the choice of food for test animals is crucial, as Dr P. M. Newberne (Massachusetts Institute of Technology) reported. His group recommended that diets for carcinogenesis testing should be standardized, so that results from different laboratories can be compared and reproduced. For this purpose they would like to see manufacturers producing semi-synthetic diets free of known enzyme inhibitors and antioxidants, and containing standardized amounts of protein, carbohydrates, vitamins and minerals.

Some of the problems besetting the hard-pressed pathologist who has to interpret the results of these various carcinogenesis tests were described by Dr W. Richter (University of Chicago), giving one of the individual contributions to the conference. Sometimes a supposed tumour turns out to be an abscess, or there are cases such as the hyperplastic rodent bladder which looks, in the microscope, like a normal primate bladder.

Considerable energy is now being devoted to the development of tests which can be carried out more quickly than those involving live animals. Dr J. A. DiPaulo (National Institutes of Health) said that some tests using a Syrian hamster cell culture system at the National Cancer Institute can be completed in ten days. Speaking for the discussion group concerned with tissue culture techniques, Dr B. Weinstein (Columbia University, New York) said that modesty is required in anticipating the outcome of tests of carcinogenicity on, as well as hamster cells, mouse fibroblasts and epithelial cells, which come principally from the rat liver. The problems of using tissue of human origin also seem to be legion, as the discussion group charged with considering this question discovered. Dr A. Freeman (Children's Hospital, Akron, Ohio) reported the recommendations that organ culture methods must be developed for testing carcinogens, and that cell banks should be established. His group also suggested that a committee should be set up to help carcinogenesis testers to obtain human tissue from inbred populations, which may be predisposed to cancer, in remote geographical areas. When it comes to obtaining the necessary evidence from the transplantation of transformed cells into donors (to establish whether they develop cancer), there are obvious objections to the use of human subjects. Dr Freeman's group therefore recommended that evidence should be accepted from xenografts, but only with concomitant and careful pathological investigation.

The general feeling of the conference, summarized by Dr D. T. Rall (National Institute of Health Sciences), was that the life-time studies of whole animals are essential to carcinogenesis testing, but that short term methods also have their function.

MEMBRANE TRANSPORT

Transduction Deductions

from our Molecular Biology Correspondent

THE study of active transport is a thriving industry at the heart of which lies a kernel of profound ignorance: it is simply not known how the hydrolysis of ATP occasions the transfer of ions through the membrane against a concentration gradient. Many facts about the process have been amassed, one of the most salient of which emerged only recently, namely that the calcium ATPase of sarcoplasmic reticulum at least can be made to go into reverse, to function, so to speak, as a motor instead of a dynamo, and synthesize ATP from ADP and phosphate, when the concentration gradient is allowed to run down. A facet which may be common to all ATPase systems is the ability to catalyse the exchange of oxygen between water and phosphate, which, it is supposed, is in some way related to a step in the ATPase mechanism. Boyer and his associates have shed some light on this dark corner, and two new articles on ATPases of quite different kinds again point to an underlying unity.

Dahms and Boyer (*J. biol. Chem.*, **248**, 3155; 1973) have examined the sodium–potassium ATPase from kidney membrane and from the electroplax of the electric eel. The preparations of these enzymes catalyse the rapid entry of ^{18}O from isotopically enriched water into phosphate ions in the medium. This is clearly related to the primary enzymatic function, for it requires potassium and is annihilated by sodium, and also ouabain, which is a specific inhibitor of sodium–potassium ATPase. It also requires the presence of magnesium. It is unaffected, on the other hand, by oxidative phosphorylation uncoupling agents, such as dinitrophenol, and it differs also from the mitochondrial system in requiring no nucleotide. Moreover there is no exchange between ATP and either water or orthophosphate.

These features render it unlikely that the water–phosphate exchange is a result of reversal of the ATPase reaction. On the other hand, the exchange reaction, when inhibited by sodium, can be set going again by addition of ATP. This points to a phosphorylation step in the mechanism, and there is also evidence that ouabain operates by stabilizing an acyl phosphate intermediate of the enzyme.

The inferences are supported by the second study (Kanazawa and Boyer, *ibid*., 3163), which concerns the calcium–magnesium ATPase of sarcoplasmic reticulum. This enzyme stimulates the same exchange of oxygen between water and phosphate in the absence of calcium, which is a strong inhibitor. Neither ouabain nor oxidative phosphorylation uncouplers affect the reaction. The turnover efficiency is greater than that of the calcium ATPase reaction by more than an order of magnitude. The calcium-dependence is strongly cooperative, and the inhibition can be reversed by chasing off the calcium with high magnesium concentrations. Exchange is inhibited by ATP and by ADP. Again, the exchange reaction can be linked to phosphorylation of the enzyme, for under the same conditions phosphorus from orthophosphate is incorporated into the protein. Calcium exerts a strongly cooperative control over this, as over the exchange reaction. These observations, it should be noted, pertain to sarcoplasmic reticulum vesicles. A monionic detergent, which leaves the ATPase activity unimpaired, stops both ion transport in the vesicles, and the oxygen exchange reaction, to which the process is evidently related.

On the basis of these results, Kanazawa and Boyer have constructed an active transport scheme, involving a conformational "pre-equilibrium" of the enzyme, with an allosteric response to calcium, for which the existence of two sites per molecule is surmised. In the state of high calcium affinity the enzyme is responsible for translocation of calcium ions. The other conformational isomer has a single magnesium site (because there is no cooperative response to magnesium), and does not bind calcium, and is responsible for moving magnesium ions across the membrane. The transport cycle contains phosphorylation and dephosphorylation steps, and the exchange of the oxygen can be accommodated in the scheme as a consequence of reversal of the reactions involving the uptake of phosphate in the presence of magnesium, and the covalent coupling of the phosphate group to a side chain, with elimination of water. The exchange reaction may thus prove to be the key to the model for active transport and related energy transduction processes, and the implications that Kanazawa and Boyer have found in it will now need to be digested by biochemists at large.

An at least superficially simpler system is the glucose transport machinery of red cells. This has served therefore as something of an archetype for pencil-and-paper models of active and passive transport. The directness of some observations and their interpretation by Edwards (*Biochim. biophys. Acta*, **307**, 415; 1973) on this system comes as an agreeable anodyne after the alarums and excursions of ion pumps. It is known that glucose transport is inactivated by exposure of the red cells to the amino group-specific reagent, fluorodinitrobenzene. What Edwards now finds is that the rate of inactivation, which must be a reflexion of the conformation or accessibility of the target protein, is modified by the presence of glucose, and differs by more than two-fold according to whether the glucose is inside or outside the cell. The regulation of the reaction is specific for glucose, and is not simply an osmotic phenomenon. The results are most easily explained in terms of two alternative states for the glucose receptors, which, Edwards suggests, may be the outward and inward-facing orientations of a carrier protein.

MARINE BIOLOGY

Fish Eggs and Larvae

from a Correspondent

A SYMPOSIUM on the early life history of fish, sponsored by a number of international organizations including FAO, ICES (the International Council for the Exploration of the Sea), SCOR (the Scientific Committee for Oceanic Research) and IABO (the International Association of Biological Oceanography) was held at the Dunstaffnage Marine Research Laboratory of the Scottish Marine Biological Association, Oban, Argyll, Scotland, from May 17 to 23.

The meeting generally accepted that high mortalities in the early stages are an important factor in determining the future brood strength and success of a year-class on recruitment to the subsequent fishery. From the results of plankton surveys on fish eggs an extensive mortality seems to occur in some species, like sole (J. D. Riley, Fisheries Laboratories, Lowestoft) and pilchard (A. J. Southward and N. Demir, Marine Laboratory, Plymouth), which cannot be explained by current systems carrying them out of the area, nor by predation. Indeed, in the case of buoyant eggs dead specimens are commonly found in the plankton hauls. It seems that in the developmental process there may be imperfections which could vary from year to year, which could be caused by maternal effects, and which could be enhanced by unfavourable environmental conditions. The extent to which eggs in delicate stages of development, for example before gastrulation, could be killed during capture has obviously been inadequately investigated and needs further study. After hatching, losses due to starvation and predation are heavy. Many larvae are extremely small and need food organisms of less than 10μ in size (B. R. de Mendiola, Institut del Mar, Lima). Attempts to quantify the nutritional status of larvae, and therefore their viability, have been attempted in the laboratory by using chemical and morphological techniques. There is some evidence that starving larvae, which are inactive and near neutral buoyancy, may be selectively sampled by plankton nets (J. H. S. Blaxter and K. F. Ehrlich, Dunstaffnage Laboratory). Predation has been studied experimentally and it is likely that unsuccessful strikes by predators may cause additional mortality because of the

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φX174 Promoter Regions Isolated

By using bacterial restriction endonucleases to cleave viral DNA molecules into sets of fragments that can be ordered molecular virologists can now obtain physical maps of viral genomes. At the same time, by using ribosomes or DNA dependent RNA polymerase to protect respectively ribosome binding sites or polymerase binding sites from nuclease attack, it has proved possible to isolate these interesting regions of various nucleic acids and a few such fragments have been sequenced. In *Nature New Biology* next Wednesday (June 20) Chen, Hutchison and Edgell report how they have combined these two techniques to isolate and localize the promoter regions, or to be more precise the RNA polymerase binding sites, of coliphage ϕX174 DNA.

Having previously analysed and ordered the fragments produced by the digestion of double stranded replicative form ϕX174 DNA with three endonucleases, Chen and his colleagues assayed the extent to which these various fragments bind RNA polymerase. They also bound RNA polymerase to intact ϕX174 DNA, digested the DNA with pancreatic DNase and isolated the pieces of DNA protected by the polymerase. These fragments were then identified by hybridizing them to fragments of ϕX174 DNA obtained with restriction enzymes.

The results of these and other experiments indicate that the ϕX174 genome has three ribosome binding sites which may not be identical. No doubt Chen *et al.* will now begin to sequence these fragments which presumably code for the initiation of transcription.

delicate integument and general fragility of larvae.

There is an almost complete lack of data on microdistribution. Most plankton nets are designed to filter large volumes of water and are not always equipped with closing devices to limit the depth of sampling. There is indirect evidence that larvae make small scale horizontal and vertical migrations and are not completely dependent on convection currents. Concentrations of larvae may be found in certain areas which suggest some sort of positive aggregating mechanism (J. M. Miller, Hawaii Institute of Marine Biology). In experimental conditions anchovy larvae will aggregate on small food patches (J. Hunter and G. Thomas, Southwest Fisheries Center, La Jolla, California) and herring larvae will perform vertical migrations which seem to have no obvious immediate adaptive value except in exploiting current systems at different levels in the water (S. Seliverstov, Polar Research Institute, Murmansk). A reshaping of ideas on larval distribution may emerge from the symposium.

Substantial knowledge on tolerance to high temperature, low oxygen, H_2S, heavy metals and other potentially deleterious factors in the environment has been accumulating in the past few years. These data are of value for assessing the losses due to entrainment in power station effluents (D. Hoss *et al*., Atlantic Estuarine Fisheries Center, Beaufort, North Carolina) and for assessing the possibilities of exploiting heated effluents to improve larval growth in aquaculture. The improvement in culture techniques in the past few years has been remarkable, especially due to work in the United Kingdom, France and the United States. New sources of small food organisms and improvements in technique mean that, given adequate finance, most fish species can be reared from the egg. The main remaining stumbling block is obtaining mature adult fish. Although some species with extended spawning periods, such as the anchovy, can be induced to mature by the use of suitable photoperiods and temperature regimes, in most species the control of the maturation process is not practised, although spawning in ripe adults can be induced by injection of gonadotropins. In the symposium, Pütter's theory was once again resuscitated by Dr J. Flüchter (Bayrische Landanstalt für Fisherei, Starnberg) and it must be admitted that the possible use of dissolved organic matter as larval food has still not been resolved. In aquaculture the use of hybrids and gynogenesis is gradually coming into vogue (C. E. Purdon, Fisheries Laboratory, Lowestoft) and the manipulation of genetic material and selective breeding are obviously destined to be profitable lines of research in the future.

GRASSLAND ECOLOGY

Land Use in the Veld

from our Plant Ecology Correspondent

It is now widely accepted that much of the grassland and savannah of Africa is not climatic climax vegetation. The combined effects of regular firing and intensive cattle grazing have imposed upon these habitats a powerful constraint which has deflected the natural course of succession. It is a matter of speculation whether these areas could have developed naturally in this way, for example, as a result of fires from lightning strikes, in the absence of human influence.

Strang (*Biol. Conserv*., **5**, 96; 1973) now describes the results of "bush-encroachment" which is currently causing problems on the high veld of Rhodesia. In this upland area the rainfall (75–105 cm) is adequate for the support of woodland and the region can be regarded as a transition zone between dry savannah to the south and forest to the north. The early European settlers of this region, however, found extensive grasslands which were maintained by frequent fires and high grazing pressures from domesticated cattle. Land use in the high veld has changed little since those days, for only about 15% of the area has proved suitable for arable agriculture. More recently, however, the intensity and frequency of fires have been reduced very considerably and this, when combined with overgrazing which is becoming increasingly common, may be the chief factor underlying the spread of scrub and woodland.

The encroachment of scrub reduces foliage production in the grasses, and is therefore detrimental to the cattle industry. Experimental clearance of this woodland has been shown to increase the carrying capacity of the veld from one beast per 8–12 ha to one beast per 3–4 ha. Strang evaluates the various methods available for scrub control and clearance and concludes that the reintroduction of intensive burning would not be an efficient means of attaining these ends. If burning takes place just once every 4 years then it effectively means that one-quarter of the grazing lands will be non-productive in any given year. The alternative methods of control, such as manual clearance or

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Haemin Effect on Protein Synthesis is Non-specific

The conclusion reached by Mathews *et al.* in their study of the specificity of the control of protein synthesis by haemin (see *Nature New Biology* next Wednesday, June 20) was foreshadowed by the results of an earlier study by Mathews in which he showed that haemin stimulates the translation of endogenous messenger RNA and EMC viral RNA, as well as globin RNA, in the ascites sub-cellular system.

The non-specific effect of haemin is even more dramatically demonstrated by this new work in which Mathews *et al.* used the rabbit reticulocyte lysate as a test system and ^{35}S-methionine to label the nascent protein.

Mathews *et al.* show that not only rabbit globin but also the chiefly larger and presumably haemin-free reticulocyte proteins synthesized in small amounts (about 10% relative to globin) in the endogenous system (lacking added mRNA) are stimulated by haemin. There is a gradient of stimulatory effect from low to high molecular weight proteins for which Mathews *et al.* propose the explanation that completion of existing polypeptide chains contributes significantly to the pool of nascent proteins analysed.

Messenger-directed synthesis of mouse globin, α and β calf lens crystallin, and EMC viral proteins in this system are all stimulated markedly by haemin. It has been suggested that haemin prevents the action of one or two different inhibitors of protein synthesis which accumulate in the lysate during incubation. Furthermore, the Cambridge group have shown previously that both the inhibitors act by preventing the association of met-tRNA$_F$ with the small ribosomal subunit before binding mRNA. In such a case it would not be surprising if the effect on translation of mRNA were non-specific. The experiments presented in *Nature New Biology* provide a good test of the suggested mechanism of action of the inhibitors, and the new results are fully consistent with this effect. Mathews *et al.* cite an article in the press in which they claim that the met-tRNA$_F$-40S ribosome complex is a true intermediate in the initiation of protein synthesis

Mathews *et al.* have thus exploded the long-standing myth that haemin (which is incorporated into haemoglobin) has a specific regulatory effect on globin synthesis. Previous workers in this field failed to carry out the appropriate control experiments before propounding this theory. The question remains whether the haemin effect has any physiological significance because, as Mathews *et al.* point out, evidence has been adduced to show that protein synthesis in HeLa cells (unlike that in reticulocytes) is resistant to inhibition by iron chelating agents. They suggest that the haemin effect, even if it does not operate *in vivo*, may be a "paradigm" for the control of protein synthesis when a general mechanism of switching on or off is required by the cell.

ring-barking, mechanical clearance and the use of arboricides, are all expensive. Strang favours an alternative to cattle ranching, that is the cropping of wild herbivores.

Game ranching offers a number of advantages over cattle grazing. In particular the wild herbivores are able to exploit the full spectrum of natural primary production rather than being dependent on certain palatable species. Most of the game animals involved are browsers rather than grazers, hence they would not be adversely affected by scrub encroachment. An important argument against this solution is the difficulty of disease control in mixed communities of wild herbivores. Perhaps a greater problem is the social upheaval which would undoubtedly result from such a radical agricultural policy —a policy which has yet to be justified economically.

ENERGY RESERVES

Bio-fuel Gauge

from a Correspondent

OF all the problems facing small homoiothermic animals living in cold places, hypothermia is possibly the greatest. This is probably one of the reasons why there is an abundance of small birds and mammals in the tropics and a scarcity in Arctic and Alpine regions. Hypothermic torpor in small animals is far from uncommon, and recently it has been shown to occur in the broad-tailed hummingbird (*Selasphorus platycercus*) of North America. What is especially interesting is the series of factors bringing about its onset.

Calder and Booser (*Science*, **180**, 751; 1973) have examined the fluctuations in temperature of hummingbird nests during incubation in the nesting seasons of 1971 and 1972. A study site at 2,900 m elevation was established in the Elk Mountains, Gunnison County, Colorado. Thermistor probes were embedded in elastomer "eggs", and the pattern of the hen's absences from the nest was recorded by interpretation of the rapid cooling spikes on the temperature trace.

Hummingbirds cannot feed in the rain, and during one particular 24-h period in June 1972 the weather deteriorated to such an extent that the incubating females missed several of the normal, periodic feeding flights. The hens of two wired-up nests lost approximately 21 and 12% respectively of their normal feeding opportunities. In an animal with a delicately balanced energy budget this reduction in energy intake can be expected to have a profound effect on subsequent behaviour. At about 0100 h in the night following this catastrophe the air temperature fell to −1.0° C, and the temperature of nest 1 began a steady decline from 31° C to 6.5° C. It then stabilized and seemed to be regulated. The temperature of nest 2 showed a similar decline and stabilized at the same level, but the decline started almost 3 h later. In both nests normal temperature was regained by 0500 h—the time at which feeding flights normally recommenced.

The chart from nest 2 showed several sharp drops immediately before the onset of hypothermia. This recalls the "test drops" made by ground squirrels when entering hibernation (Sturmwasser, *Am. J. Physiol.*, **196**, 8; 1959), but they also resemble normal brief nest departures. It is therefore possible that the hen from nest 2, sensing its energy reserves were insufficient for the weather conditions, tried to feed during the night in an attempt to preclude the need for torpor.

The fascination of these observations lies in the fact that the periods of hypothermia endured by the two hens started at different times but ended simultaneously. The length of time spent in torpor seemed to be correlated with the reduction in feeding opportunities (21 and 12%) presented to hens in the previous daytime. This is not so strange, for it has long been known that the duration of torpor induced in laboratory rodents is correlated with food intake (Barthomew, *Ecology*, **50**, 705; 1969). What is curious is that the reduction in number of occasions for energy intake acts as a fuel gauge to "inform" the bird of the precise moment when it should enforce the necessary economies. These economies seem to be withheld until the last possible moment, doubtless in the hope of a sudden rise in air temperature.

Cooling seems not to damage birds' eggs, but it does increase incubation time. Perhaps frequent resort to torpor is an important factor in the long incubation periods seen in hummingbirds nesting high in the Andes.

MATERIALS SCIENCE

Analysis of Surfaces

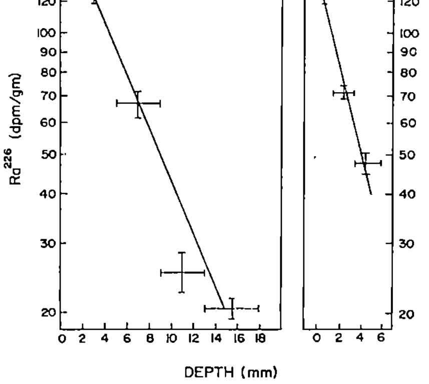
from our Materials Science Correspondent

ARGUABLY the most productive new instrument in materials science since the war has been the electron microprobe analyser. This instrument, which depends on the emission and analysis of characteristic X-rays, stimulated by a minute exploring beam of electrons impinging on a selected point on a polished specimen, has transformed the study of solidification, phase transformations, diffusion and corrosion. Its essential feature is that the chemical ana-

How Do Nodules Grow in Freshwater?

THE question how ferromanganese nodules grow in freshwater is considered by Krishnaswami and Moore in *Nature Physical Science* next Monday (June 18). They discuss the insight cast on this problem by their study of two nodules, one a sphere 2 cm in diameter from Lake Alstern, Sweden, and the other a saucer shaped concretion about 2 cm thick from Oneida Lake, New York.

If the growth rates of the deposits are in the range 100 to 1,000 mm per 10^3 yr, ^{228}Ra (half life 6.7 yr) and ^{210}Pb (half life 22.3 yr) should be ideal for measuring the rate of accretion. Surprisingly, however, the appropriate analysis of these two nodules yields accretion rates of only 1 to 3 mm per 10^3 yr, with ages around 6,500 yr, similar to those reported for iron–manganese nodules from Lake George, New York.



Variation of ^{226}Ra with depth in the two nodules.

Taken at face value, then, these data suggest that sediment around the nodules accumulates 1,000 times faster than the nodules grow. How, then, do the nodules maintain their position at the sediment/water interface? Perhaps active currents play a part in keeping the nodules exposed, which would also explain why nodules are abundant where wave action is strong and fine sediment absent. Or perhaps there is another interpretation.

Krishnaswami and Moore point to three possible sources of error in their approach: first, the ^{226}Ra concentration in the lakes may be increasing; second, radium may migrate to the outside of the nodules during growth (although no such effect is observed for barium); and third, ^{226}Ra may be diffusing inwards, producing an apparent radioactive decay with depth. But if the nodules are as old as they seem to be, they should certainly provide valuable information on the trace element history of the water in which they formed.

lysis is localized, whereas X-ray fluorescence analysis of the longer established kind, which depends on excitation by hard incident X-rays, averages the composition over several square centimetres. The wheel is, however, coming full circle with the introduction of macroscopic X-ray analysis by electron excitation, which in its latest form allows measurements of elements down to boron (Price, *Metals and Materials,* **7,** 140 ; 1973). The use of greatly improved detectors in conjunction with non-dispersive energy analysis has steadily improved sensitivity as well as light-element detection in both microscopic and macroscopic variants of electron-excited X-ray analysis.

Electron probe microanalysis used to be regarded as a form of surface analysis, but in fact the effective depth over which composition is integrated is of the order of 1 μm. True surface analysis, by contrast, requires physical methods that explore a single surface monolayer, or ~1 nm depth at most. There is now a very varied array of techniques available—the emission of photons, electrons, ions or neutral particles can be stimulated by any of these four, or by heat or an intense electric field for good measure.

More than twenty permutations of stimulation/emission exist, each with its own distinct physical theory, sensitivity, speed, depth of measurement, convenience and limitations. Electron-excited X-ray (that is, photon) emission is merely one among many. The onlooker who is concerned with using these techniques rather than developing them may be forgiven for being bemused. Most survey articles, of which there is no shortage, fasten on one technique to the exclusion of all others. For this reason, a new survey entitled "New Developments in the Surface Analysis of Solids" does special credit to the author, Benninghoven of the University of Cologne (*Appl. Phys.,* **1,** 3 ; 1973). (The journal is a new English-language venture by Springer–Verlag which replaces the excellent German-language *Zeitschrift für angewandte Physik,* which ceased publication at the end of 1971.)

Benninghoven is a specialist in surface studies and his name is especially associated with secondary-ion mass spectrometry (SIMS), of all methods the one best suited to analyse a single surface monolayer. He describes in some detail in his survey the advantages of SIMS (including the ability to follow chemical reactions at the surface as they progress), but devotes attention also to the various forms of secondary-electron spectroscopy (including both Auger electron (AES) and photoelectron spectroscopy, the latter usually known by its originator's designation of ESCA —electron spectroscopy for chemical analysis), and even to the very novel technique of "soft X-ray appearance potential spectroscopy", which depends on the sudden appearance of characteristic X-ray emission lines as the energy of bombarding electrons is gradually increased. Benninghoven explains the essential physics of each technique without getting side-tracked along fascinating but inessential detours (such as the mechanism of surface-ion emission in the sputtering situation) and shows how emission of different types can be reliably distinguished, how the state of chemical combination can be assessed by some techniques but not by others, how and why the effective depth of analysis varies between techniques, which are best for analysing light elements including hydrogen, and how sensitivity varies. The examination of states of chemical combination is particularly important in many applications, but the very power of this technique (see Siegbahn, *Endeavour,* **32,** 51 ; May 1973) has led to its use predominantly in "bulk situations" rather than in surface studies hitherto.

Another technique, related to SIMS, is ion-scattering spectrometry (ISS). Here an exploring beam of incident low-energy ions, often He or Ar, is scattered from the surface layer: the energy distribution of the same ions is measured after scattering, and simple calculations based on Rutherford scattering theory permit deductions as to the masses of the atoms in the surface layer. This technique is outlined in a clearly and comprehensively written survey of trace analysis techniques for solids (both for surfaces and for bulk solids) by Kane and Larrabee (*Annl. Rev. Mat. Sci.,* **2,** 33 ; 1972). This review can with benefit be read in association with Benninghoven's.

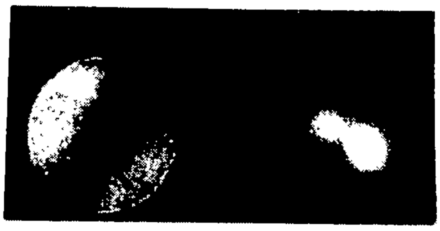
Overall, Benninghoven's most useful survey leaves the impression that SIMS, a technique not yet widely familiar, has particular promise as a highly sensitive and discriminating method of identifying and analysing surface species.

New Techniques for Ionospheric Research

The simplest model of the ionosphere is one in which the electron concentration at a given height does not vary with position. It is a good first approximation in most situations, but for many years ionospheric workers have been considering the ways in which it fails to represent the real ionosphere; for example, spatial irregularities of various kinds are often found, and there have been attempts to study their structure and movements, often in the hope of obtaining information about winds or waves in the ionosphere.

Two Australian communications in *Nature Physical Science* next Monday (June 18) each describe a different improvement in technique to study such phenomena. Both use large arrays of aerials which can achieve high angular resolution in the reception of radio waves at frequencies from 2 to 6 MHz, but differ greatly in their method of handling the received signal. Briggs and Holmes use for each aerial a superheterodyne receiver connected to a transducer which forms part of an acoustic array—an analogue of their radio receiving system—under water. If the acoustic sources lie on a plane surface they would simply produce an acoustic field analogous to the original electromagnetic field, and this is not immediately useful. The sources are, however, arranged on a spherical surface in such a way that a plane wave falling on the aerials gives rise to an acoustic wave focused on an image plane. The position of this focused point is a measure of the direction of arrival of the original radio wave. A set of transducers on this image plane energizes an array of light-emitting diodes to make visible the directions of all incident radio waves.

The figure here shows on the left the distribution of radio wave intensity over the array and on the right the angular distribution obtained from it by the acoustic technique; two modes of propagation can be distinguished. The method can also be used to follow the reflexion points of waves from a fixed transmitter and thus to study irregularities. The transmitter need not be pulsed nor indeed controlled by the experimenter, so uses in radio astronomy and in direction-finding are quite possible.



The method of Brownlie *et al.* is more limited in application. A beam is formed by transmitting pulses from one arm of a cross and receiving them on the arm at right angles, and is steered by phase shifters under computer control. By rapid scanning a display can be produced showing the angular distribution of received radio waves. The technique is clearly very suitable to the study of highly irregular ionospheric structures such as "spread-F". Similar systems have been constructed previously for different purposes, but with receiving beams only (for example, Morris *et al., Proc. Instn elect. Engrs,* **110,** 1569 ; 1963).

EARTHQUAKE PREDICTION

Precursory V_p/V_s Changes

from our Geomagnetism Correspondent

MORE than a decade ago, Kondratenko and Nersesov (*Trudy Inst. Fiz. Zemli*, **25**, 130; 1962) reported that the velocity of crustal P waves increases after moderately large earthquakes in the Tadjikistan region of southern central Asia. They had analysed more than 800 travel-time curves from foreshocks and aftershocks of earthquakes with magnitudes 5 or greater and occurring during the period 1958–1961, and had concluded that the average P wave velocity was 5.3 km s^{-1} prior to main shocks and 6.3 km s^{-1} afterwards. This was the first report of a variation in seismic velocity associated with earthquakes; but unfortunately it was not translated from the Russian and so apparently remained unknown in the west (and even more surprisingly in Japan) until Savarensky (*Tectonophysics*, **6**, 17; 1968) reviewed the data in English.

There can be little doubt that had this discovery been made known at the time outside the Soviet Union, it would have attracted considerable attention, especially in Japan where earthquake prediction research had long been a subject of great concern. The sheer magnitude (more than 15%) of the reported velocity change of the P waves would have ensured attempts to repeat the analysis elsewhere. Rikitake (*Earth Sci. Rev.*, **4**, 245; 1968), for example, was later to imply doubt about the validity of the Russian data on the grounds that a P wave velocity change of 15% would correspond to a 30% change in elasticity. As it was, the delay in the communication of the Russian results was compounded by the fact that, throughout the 1960s, seismic velocity was not apparently among the many parameters independently studied from the point of view of earthquake prediction.

Following Savarensky's report and other reports of Russian work (see, for example, Nersesov *et al.* in *The Physical Basis of Foreshocks*, Moscow, 1969), the subject was taken up by United States seismologists. In 1969, Semenov (*Izv. Acad. Sci. USSR Phys. Solid Earth*, **4**, 245; 1969) had shown that prior to earthquakes in the magnitude range 3–5, the P wave–S wave velocity ratio (V_p/V_s) decreased by about 6% and then increased again to about its normal value just before the onset of the main event. Aggarwal *et al.* (*Nature*, **241**, 101; 1973) then reported a similar phenomenon involving V_p/V_s) decreases of up to 13% for earthquakes in New York in the magnitude range 1–3. But these changes all involved relatively small events; and it was clearly desirable to determine whether similar precursors apply to larger earthquakes. Proof that they do has now been supplied by Whitcomb *et al.* (*Science*, **180**, 632; 1973) in respect of the 1971 San Fernando earthquake (magnitude 6.4). This report from Whitcomb and his colleagues, coming at a time when many people have become discouraged by the apparent lack of progress in the highly intractable problem of earthquake prediction, is likely to become a landmark in the subject, for not only does it present convincing evidence for the phenomenon concerned, but it has also been possible to view an earthquake precursor in terms of a realistic physical model.

The facts are easily stated. Whitcomb *et al.* have used P and S waves from nineteen earthquake sources during 1961–1970 to show that the V_p/V_s ratio in the San Fernando epicentral region had an average value of 1.75 up to 1967 but decreased suddenly by 10% in the middle of that year and then gradually increased to about its normal value at the time of the 1971 shock. When the P and S wave velocities are examined individually both are found to change in the same sense as the V_p/V_s ratio, but the V_p variation is the greater and the variation in V_s is little greater than the uncertainties of measurement. Thus, contrary to some previously stated beliefs, it is V_p which accounts for the change in V_p/V_s. This is an important result, not only because it leads directly to a possible physical explanation of the precursory variation but because of its practical consequences. Man-made explosions produce chiefly P waves, any S waves generated being difficult to measure. The new result thus opens the way to the use of artificial sources to detect precursory V_p/V_s changes, rather than having to depend on the vagaries of earthquakes.

The second important result concerns the interval between the sudden V_p/V_s decrease and the onset of the earthquake, which in the San Fernando case is 3.5 yr. Combining this result with data from previous studies, Whitcomb *et al.* show that, over the magnitude range 0–6.4 at least, the precursory interval increases logarithmically with earthquake magnitude. Extrapolation of this variation (the validity of which will need testing by future studies) suggests that a magnitude 7 event would have a precursory interval of 8 yr and that a magnitude 8 event would have an interval of 40 yr. Clearly no great reliance should be placed on these absolute figures at this stage; but what is quite certain is that the precursory interval increases with magnitude. By contrast, the precursory V_p/V_s decrease is independent of magnitude.

Whitcomb and his colleagues then go on to show that all of these results may be accommodated, both qualitatively and quantitatively, by a physical model derived from the known behaviour of fluid-filled, porous media—in particular, the phenomenon of rock dilatancy, or the increase of volume resulting from a change in shape. In the field, the change in shape is thought to be caused by shear stresses associated with regional tectonic strain. Assuming the pores and cracks in the rock to be initially saturated with fluid, the dilatant volume increase will lead to a decrease in pore fluid pressure, and if the dilatancy is large enough the pore and crack volume will ultimately exceed the fluid volume. In this undersaturated stage the enlarged rock voids will partially contain vapour, resulting in a reduction of the overall bulk modulus. This produces a decrease in V_p but little change in V_s and thus accounts for the drop in V_p/V_s.

The effect of the reduction in pore fluid pressure is to increase the fracture strength of the rock and thus delay the onset of the earthquake. Fluid flow from outside the dilatant volume, however, gradually returns the permeable rock to its saturated state, producing a gradual increase of V_p/V_s and a gradual decrease in fracture strength until an earthquake occurs. The actual variations in V_p and V_s during these processes depend on the range of velocities in the rock between the saturated and undersaturated states. This range is independent of earthquake magnitude, and so the drop in V_p/V_s must also be independent of earthquake magnitude. On the other hand, the rate at which the rock returns to the saturated state depends partly on the permeability of the rock and the availability of fluids but also on the dilatant volume. The dilatant volume, in turn, must depend on the amount of stored mechanical energy which presumably determines the magnitude of the final earthquake. The precursory interval—the time taken for V_p/V_s to return to its normal value—should thus depend on the earthquake magnitude, the greater the magnitude the longer being the interval.

It is this marriage of observation and physical theory which makes the report from Whitcomb and his colleagues particularly important. Thus, in spite of the historical accident which delayed the wider communication of the original discovery, the V_p/V_s precursor now emerges as probably the most promising of all possible earthquake precursors. As Whitcomb *et al.* point out, however, there are other phenomena (such as electrical conductivity) associated with dilatancy. Some of these have already been studied with a view to earthquake prediction, but such studies may now become more significant.

Contemporary Population Densities and Human Health

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High population densities are not associated with high mortality when socioeconomic factors are controlled.

HIGH population densities are commonly asserted to be one of the chief causes of crime, mental breakdown, stress-related illness, and other contemporary problems (for example, refs 1-3). This assertion has been based in part on studies of the social pathology that develops in caged rodents at high population densities (for example, refs 4 and 5). Although it may be intriguing to extrapolate from the behaviour of rats and mice physically trapped in a very simple and apparently boring[6] cage to the behaviour of humans economically trapped in a decaying urban area, no scientific validity can be assumed for this extrapolation. We do not even know how high densities would affect rodents in complex environments that would provide the opportunities which natural environments provide for exploration and for avoiding aggressive interactions. Furthermore, the great differences between the behavioural repertoire, social structure, and physical surroundings of the rodents in the experiments and those of people in the cities make it unwarranted to extrapolate from rodents to humans on the basis of a single similarity: that both groups are living at densities many times higher than have been typical of their species.

Careful studies on humans have failed to provide convincing evidence that contemporary population densities have significant harmful effects. (Brief reviews and critiques may be found in refs 7-9.) There is no simple or reliable relationship between changes in population size and the frequency and intensity of wars[10]. Brief exposures to extremely high densities have a variety of effects which are relatively small and not all harmful[9,11].

Many discussions of the long-term effects of high densities on humans have failed to distinguish between the effects of density *per se* and the effects of other factors, such as poverty, which are commonly associated with high density. Winsborough[12], Schmitt[13], and Galle *et al.*[8] attempted to separate the effects of density from the effects of other socioeconomic factors by using partial correlation analysis. For example, they computed the partial correlation coefficient between density and mortality rate with income partialled out. This coefficient was interpreted as a measure of the association between density and mortality with income "held constant". Although this is a very common interpretation of partial correlation coefficients, it is an invalid one. This coefficient is only a measure of the amount of information which density gives about mortality above and beyond the information which income gives about mortality. Partial correlation analysis cannot be used to compare the relative effects of income and density *per se* on mortality. As discussed more fully by R. M. F.[14], this common misinterpretation of partial correlation coefficients is based on a hidden circularity: one must make implicit assumptions about the presence or absence of causal relationships when in fact it is precisely these relationships which are to be discovered.

In addition there are serious inadequacies in the data used by Schmitt, Winsborough, and Galle *et al.* Schmitt[13], in his analysis of the correlates of density for Honolulu census tracts, did not adequately correct for the effects of age structure on his health measures, and used very crude measures of education and income as the controlled factors in his partial correlations. Winsborough[12] and Galle *et al.*[8], in their analyses of the correlates of density for Chicago community areas, used the number of residents per square mile as a density measure. Some of the least dense areas by this measure were highly industrial or commercial, often with very high populations during business hours. In nearly half of the community areas, more than one-third of the land area was devoted to nonresidential use. The number of persons per square mile was therefore a poor measure of residential density.

We present here two analyses of long-term effects of population density on human health. We control for the effects of other socioeconomic factors by comparing areas where these other factors do not differ, thus obviating the need for partial correlation analysis. Both analyses indicate that, within current ranges, population density, measured as the number of persons per area, does not have large effects on human health.

Correlates of Density in Chicago

Chicago has been divided into seventy-five internally homogeneous community areas, from which we selected ten, which in 1960 were similar with respect to eleven socioeconomic characteristics. (Data are for community areas 4, 15, 16, 17, 18, 19, 20, 25, 66 and 71 and are from ref. 15, except land areas, which are from ref. 16.) In each of these areas, less than 0.7% of the population was non-white or Puerto Rican; median family income was between $7,306 and $8,014; median years of school completed by persons over twenty-five varied from 9.6 to 11.2; 2.4–3.1% of the male labour force was unemployed and 35–52% were white collar workers; 45–55% were first or second generation immigrants to the United States; 56–67% of the females and 65–72% of the males aged fourteen and over were married; 23.5–30.5% of the population was less than eighteen years old; 1.4–4.0% of the housing was substandard; persons per household divided by median rooms per household was between 0.59 and 0.65; and between 8 and 25% of the land in each area was part of regions of 100 acres or more which were used for predominantly industrial and commercial purposes.

This sample of ten socioeconomically homogeneous community areas had densities ranging from 11,800 to 19,500 persons per square mile. There was also considerable variation in three related characteristics: proportion of housing units in structures with more than two dwelling units (6.5–68.5%); proportion of housing units occupied by their owners (28.0–80.5%); proportion of people living in the same quarters they occupied 5 years previously (39–51%). Community areas with higher densities had more multi-family dwellings, less home ownership, and greater geographical mobility (all rank-order correlations ≥ 0.9).

The rank-order correlation of density with age-standardized mortality is so low that it is more plausibly interpreted as due to sampling variation than as evidence of an actual relationship between density and health (Table 1). A graph of the data showed that there was no curvilinear relationship between density and mortality.

Juvenile delinquency and mental hospital admission rates had large and significant rank-order correlations with the four related factors: high population density, high multiple dwelling units, high mobility, and low home ownership. We submit the following as the most plausible interpretations of these correlations: (1) higher density leads to increase in social pathology, specifically mental breakdown and juvenile delinquency; (2) home ownership leads to less pathology; (3) people with better physical and mental health have more success in becoming home owners; (4) pathology is increased by some aspect of multi-unit dwellings such as living above the ground floor or more disturbance from neighbours; (5) disruption of social relationships by more frequent changes in residence results in greater pathology; (6) less healthy and stable people move more often, so more mobility into an area means more pathology there; (7) people in high density regions are less tolerant of deviant behaviour and therefore more likely to turn over deviants to institutions such as mental hospitals or the police.

At present, we have no data on which to base a choice among these hypotheses, and indeed several of them may be valid simultaneously. The fourth hypothesis receives some support from Mitchell's observation that adults living above the ground floor in Hong Kong residences had higher hostility scores and more psychosomatic symptoms[19]. Some evidence in favour of the fifth hypothesis comes from previous studies in Chicago[17] and Baltimore[18]. Lander's analyses showed that juvenile delinquency is highest in Baltimore census tracts that have the lowest owner-occupancy of dwellings and the most racial mixing (that is, intermediate proportions non-white). He interpreted this to mean that "anomie" or social disruption is an important cause of juvenile delinquency.

Although we are unable to choose among the seven hypotheses proposed above, we do feel that any other hypotheses we have been able to formulate are less probable than these are. For example, the pathologies at higher density cannot be attributed to more persons per room, since there was a slight tendency toward fewer persons per room at high density (presumably related to the slightly lower proportion of children). Similarly, the pathologies at higher density are probably not due to differences in education because higher juvenile delinquency, mental hospital admission, and mortality were correlated with more, not less, education in this sample of limited socioeconomic variability. None of the other socioeconomic factors was correlated with the three health measures.

If it should eventually be shown that the first of the seven hypotheses is correct and that density is a cause of juvenile delinquency and mental hospital admission, then it would be useful to have an estimate of the quantitative significance of density as compared to other causal factors. Unfortunately, as is discussed by R. M. F.[14], this cannot be determined from the existing data since density is strongly correlated with the other factors.

In summary, as there is little or no correlation of mortality with density, it seems probable that density *per se* does not have a large effect on physical health. (Admittedly, we are not sure how long each person had experienced the density of a particular community area, for almost half the families changed residences between 1955 and 1960, mostly within Chicago.) Higher density was correlated with higher mental hospital admissions and higher juvenile delinquency, but this correlation may well have been due to the fact that higher density was part of a syndrome including a higher proportion of buildings with more than two dwelling units, greater geographic mobility, and lower home ownership.

Density and Specific Diseases

In our second analysis of correlates of density, we have compared age-specific mortality rates for pairs of countries matched for *per capita* GNP and health care facilities. Obviously, the matching of socioeconomic factors is not nearly so precise in this second analysis as it was for the Chicago community areas. Also, the density measures conceal a good deal more heterogeneity. The differences in average density are, however, much larger, and the populations are large enough to allow us to make comparisons of mortality rates for specific causes and for the two sexes separately.

We chose pairs of countries for which one country was more than twice as dense as the other and also more highly urbanized[20], and for which both countries had similar literacy, income, and health facilities[21] (Table 2). We used only countries with reliable vital statistics (as evaluated by the United Nations), more than 8,000 deaths annually for each sex, and less than 13% of deaths in the category "senility without mention of psychosis, ill-defined, and unknown causes", because this category tends to be used as a catch-all in poor statistical systems. For each country, we computed age-specific death rates by sex for total mortality, and for deaths due to twenty-two selected causes which were of special interest either because of their large magnitude or because of their relationship to the psychological condition of the individual. (Age-specific death rates were calculated from deaths[20] and population[20,22] by 5-year age groups, except for England and Wales, by 10-year age groups.) The Wilcoxon matched-pairs signed-ranks test was used to compare age-specific death rates for total mortality and for each cause for each pair of countries. This non-parametric test evaluates the following null hypothesis: for two countries and for a particular cause–sex combination, the pairs of death rates for corresponding age groups are the same except for non-systematic deviations. The test emphasizes the consistency of the differences at different ages rather than the magnitude of such differences. Thus, rejection of the null hypothesis implies that the inhabitants of one country experience a higher mortality than the inhabitants of the other across much of the age-structure.

Table 2 displays the pairs of countries arranged in order of decreasing differences in density, and for each pair shows the density and socioeconomic measures, and the results of the comparisons of mortality for males and females using the Wilcoxon test. Out of the fourteen comparisons of total mortality, nine were not significantly different; in three comparisons, the high density country had higher mortality, whereas in two, the low density country had higher mortality.

Table 1 Correlations of Housing Variables with Health Measures in Chicago, 1960

	Density (persons per square mile)	Multiple dwellings (% of housing units in structures with more than two units)	Home ownership (% of housing units owner occupied)	Geographic mobility (% of persons over five who lived in a different house in 1955)
Male juvenile delinquents as a proportion of male population, aged 12–16	0.72	0.74	−0.69	0.77
Age-standardized rate of admissions to mental institutions in Illinois	0.63	0.78	−0.76	0.74
Age-standardized death rate	0.19	0.54	−0.50	0.44

Spearman rank order correlation coefficients between health and housing variables were calculated for ten socioeconomically similar community areas. Density and housing variables are highly intercorrelated. For a one-tailed test and $N = 10$, 5% and 1% levels of confidence are attained, respectively, by coefficients of 0.56 and 0.75.

Malignant neoplasms, bronchitis, and ulcers were the three causes of death most consistently higher in the high density country. Tuberculosis, vascular lesions of the central nervous system, and accidents other than motor vehicle accidents were most consistently higher in the low density country. The causes listed in the middle of Table 2 showed no consistent relationship to density. (We have included in this middle group causes for which several consistent differences have been found, but in which France was the less dense country in almost all these cases. It was unfortunate that France was the low density country in three out of seven comparisons, but we were unable to find other pairs of countries that fit the specified criteria.) Note that homicide and suicide are among the causes of death which are not consistently related to density.

As in the first analysis, the chief conclusion seems to be that density does not have large effects on human health. The statistical tests were repeated for the younger and older age groups separately and revealed even fewer consistent differences than the tests for the whole population. Visual inspection of the graphs of age-specific death rates for each cause, sex, and pair of countries did not reveal any consistent differences which were not apparent from the statistical tests. We also computed age-adjusted death rates, which contain much less information than age-specific rates. In each of the 172 cases for which the Wilcoxon test showed a significant difference in the age-specific death rates, the age-adjusted rates differed in the same direction.

It is possible that some differences between high and low

Table 2 Comparisons of Age-specific, Cause-specific Death Rates for Countries with Similar Socioeconomic Conditions but Different Densities

Socioeconomic characteristic	Hong Kong/ Taiwan		England and Wales/ New Zealand		Netherlands/ Norway		West Germany/ Norway		England and Wales/ France		Netherlands/ France		West Germany/ France	
Density (persons km^{-2})	3,770/360		320/10		375/12		233/12		320/91		375/91		233/91	
Population/1,000 acres of agricultural land	98,000/10,000		1,976/170		4,855/3,420		3,631/3,420		1,976/1,288		4,855/1,288		3,631/1,288	
% in cities with population more than 100,000 (city proper)	NA/32		41/17		32/23		30/23		41/19		32/19		30/19	
Gross domestic product *per capita* (US $)	313/288		1,700/1,885		1,610/1,988		1,746/1,988		1,700/1,984		1,610/1,984		1,746/1,984	
Population per physician	2,320/2,330		870/830		860/790		630/790		870/850		860/850		630/850	
Population per hospital bed	280/1,040		100/100		130/110		90/110		100/100		130/100		90/100	
Cause of death for comparison of age-specific death rates	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Total mortality		↓↓↓					↑↑↑	↑↑↑			↓↓↓			↑↑↑
Malignant neoplasms	↑↑↑	↑↑↑			↑↑	↑↑↑	↑↑↑	↑↑↑	↑			↑↑↑		↑↑↑
Bronchitis	↓↓↓	↓↓↓	↑↑		↑↑↑	↑↑	↑↑↑	↑↑↑	↑↑↑	↑↑↑	↑↑↑	↑↑↑	↑↑↑	↑↑↑
Ulcer of stomach and duodenum	↓↓↓	↓↓↓	↑↑↑	↑↑	↑↑↑		↑↑↑		↑↑↑	↑↑	↑↑↑	↑↑↑	↑↑↑	↑↑↑
Arteriosclerotic and degenerative heart disease	↑↑↑								↑↑↑	↑	↑↑↑	↑↑↑	↑↑↑	↑↑↑
Congenital malformations		↑							↑↑↑	↑↑↑	↑↑↑	↑↑↑	↑↑↑	↑
Hypertension with heart disease	↑				↓↓			↑↑↑	↑	↑	↑	↑	↑↑↑	↑↑↑
Motor vehicle accidents			↓↓↓		↑↑↑	↑↑↑	↑↑↑	↑↑↑						
Pneumonia	↑↑↑		↑		↓↓↓	↓↓↓			↑↑↑	↑↑↑			↑↑↑	↑↑↑
Benign neoplasms					↑↑↑	↑↑↑	↑↑↑	↑↑↑	↓↓↓	↓↓↓				
Anaemias	↓↓	↓↓↓							↑↑↑	↑↑↑			↑↑↑	↑↑↑
Homicide							↑↑↑				↓↓↓		↑↑↑	↑↑↑
Diabetes mellitus				↓										↑↑↑
Syphilis and its sequelae			↑			↑↑↑		↑↑↑			↓	↓↓	↓	
Suicide	↓↓↓					↑↑↑	↑↑↑	↑↑↑	↓↓↓		↓↓↓	↓↓↓		↑↑↑
Nephritis and nephrosis	↓↓↓	↓↓↓				↑						↑		
Chronic rheumatic heart disease	↓			↑↑	↓	↓↓	↓↓↓	↓↓↓	↑↑↑	↑↑	↑↑↑	↑↑↑	↓↓↓	↓↓↓
Cirrhosis of liver	↓↓↓	↓↓↓	↓				↑↑↑	↑↑↑			↓↓↓	↓↓↓		
Other diseases of heart	↑↑		↓↓↓		↑↑↑	↑↑↑	↑↑↑	↑	↓↓↓	↓↓↓	↓↓↓	↓↓↓	↓↓↓	↓↓↓
Senility without psychosis and ill-defined and unknown causes					↓↓↓	↓↓↓			↓↓↓	↓↓↓	↓↓↓	↓↓↓	↓↓↓	↓↓↓
Tuberculosis		↓↓↓		↓	↓↓↓		↑↑↑	↑↑↑	↓↓↓	↓↓↓	↓↓↓	↓↓↓		↓
Vascular lesions affecting central nervous system		↓↓↓			↓↓↓	↓↓↓					↓↓			
Accidents other than motor vehicle	↓↓↓		↓		↓			↑↑	↓↓↓		↓↓↓	↓	↓↓↓	

For the mortality comparisons a group of arrows indicates that the age-specific death rates for this comparison are sufficiently different that the probability is less than or equal to 0.05, 0.02 or 0.01 (for one, two and three arrows respectively) that this difference could be due to sampling variations. Groups of arrows pointing up indicate that the denser country has higher death rates for that cause and groups of arrows pointing down indicate that the denser country has lower death rates for that cause. The data are for 1966 or 1967 with very few exceptions where it was necessary to use data from slightly earlier years.

density countries were concealed by methodological problems. Although we tried to equalize medical care, several of the high density countries may have better medical care: there are more hospital beds per capita in Hong Kong than in Taiwan; there may be more equitable access to medical care in the Netherlands and in England and Wales than in France. Such methodological problems would not, however, be sufficient to obscure the major differences in death rates which result from significant differences in education and income[23,24]. Thus, at the very least we can conclude that the effects of density on health are much less than the effects of several other socio-economic factors.

Higher air pollution in more dense, more urban countries may be a major cause of the higher death rates due to bronchitis and lung cancer[25] (the latter of which is a significant factor in the higher malignant neoplasm rates). It is obviously easier to attain low levels of air pollution in smaller, less dense aggregates of population, but current levels could be much reduced, even in high density regions, by better use of existing and foreseeable technology[26,27]. Higher lung cancer is apparently also related to more migration[25] and more disruption of social groups[7] in cities.

Implications

For ten socioeconomically similar community areas in Chicago, mortality is not correlated with population density. High mental hospital admission and juvenile delinquency are, however, associated with a syndrome of higher population density, more multiple-unit dwellings, less home ownership, and more mobility.

When countries matched for income and health care are compared, total mortality is not consistently related to population density. Cancer, bronchitis, and ulcer mortality are higher in denser, more urban countries, but deaths from other causes are lower.

Thus, in our two analyses, high population density is not strongly correlated with high mortality. It seems that, within current ranges, density *per se* is not a significant cause of poor physiological health. Our study has not revealed convincing evidence of effects of population density on mental health and crime, but our methods have not been adequate to exclude this possibility. Although it would be of interest to explore further the relationship of density to other variables, it seems clear that public policy to improve health should focus directly on more well-established problems such as poverty, malnutrition, inadequate education and health care.

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Prospects for Fusion Research in Britain

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More money is to be spent in Britain on fusion research in the next few years, but in the longer term international collaboration, for example within Europe, will become increasingly important.

It is now well known that matter may be converted into energy when atomic nuclei interact in such a way that their final mass is less than their mass at the start of the reaction. Broadly speaking, the largest amounts of such nuclear energy are released either when the very heaviest nuclei in nature, such as uranium, divide into parts or when the very lightest, such as hydrogen, fuse together. The first of the two processes, the nuclear fission of heavy elements, is the basis of all the nuclear power stations at present working or under development. The second, the nuclear fusion of light elements, has so far been used to produce energy on a large scale only in thermonuclear explosions.

At the time of the development of the thermonuclear bomb, a search for a controlled method of releasing fusion energy was begun independently in the United States, the USSR, and Britain with the aim of finding a further large-scale source of energy, to add to the opportunities already being created by the controlled release of fission energy. A fusion source of energy would have a virtually unlimited world supply of fuel represented by the relatively abundant heavy isotope of hydrogen, deuterium, present in, and easily separated from, seawater.

In order to achieve fusion reactions, temperatures of the order of hundreds of millions of degrees are required. As materials at these temperatures exist only as plasma, that is, a gas of positively charged atoms and negatively charged electrons, the chief emphasis to date has been on the search for a way of producing and then confining within a restricted space such plasmas for a long enough time to yield a useful net gain of energy. Because of the temperatures involved, a non-material method of isolating the plasma is required. So far, the most likely means of successfully confining plasma has seemed to be the use of magnetic fields and from the start the search has been devoted largely to studying the behaviour of plasmas in different configurations of such fields. Very recently, however, the possibilities of the inertial confinement of very dense plasmas generated from condensed fuel irradiated with a laser have attracted some attention—and this possibility is briefly discussed later in this article.

History

The easiest way to produce plasmas at high temperature seemed to be by using high current electrical discharges through low pressure gas, and hence research into the possibilities of producing thermonuclear conditions in such discharges was begun in Britain at three universities in about 1947. This continued until 1951 when the work was transferred to the Atomic Energy Research Establishment (AERE) Harwell and to AEI, Aldermaston Court. Work also began at the Atomic Weapons Research Establishment (AWRE) Aldermaston. About the same time, fusion work was also started in the United States. All this work was classified and guarded by security restrictions—as was most atomic energy research in those days.

The work remained classified for some years until, in a lecture at Harwell in 1956, Academician Kurchatov disclosed the results of research on pulsed high current discharges in deuterium, carried out in the USSR. Later that year, full cooperation on fusion research was agreed between Britain and the United States. This was followed in 1958 by declassification of fusion research in Britain, the United States and the USSR and the work of all countries was reported in detail at the Second Geneva Conference. Perhaps the most significant outcome of the conference was the wide appreciation that further progress depended in large measure upon a more fundamental understanding of plasma behaviour.

Nevertheless, the late 1950s were a time of great optimism for fusion and in 1958 when Zeta, the first substantial toroidal device, produced nuclear reactions at a rate "not inconsistent with thermonuclear production", it was all too easy to conclude that success was at hand. Sadly, further work soon showed that this was a false dawn: the reactions were, it seems, produced not by a true temperature effect, but by a small percentage of the deuterons being accelerated by the intense electric fields generated inside Zeta by plasma instabilities. These plasma instabilities themselves resulted in a poor confinement, with confinement times of less than 100 μs.

At about this time, the need to broaden the scientific base of fusion research led the United Kingdom Atomic Energy Authority (UKAEA) to concentrate its fusion research into a new laboratory at Culham with the aim "to establish whether or not controlled fusion reactions in light elements can be achieved to provide a new source of nuclear power". It was clearly recognized that the principal problem then facing fusion research was the difficulty of obtaining sufficient understanding to suppress plasma instabilities and thus to confine the plasma. The new Culham Laboratory was therefore oriented to work on this broad problem from the outset. The moves from Harwell and AWRE Aldermaston to Culham were completed in 1964. The work at AEI Aldermaston continued under a UKAEA contract until 1963, when it was stopped by the closure of this industrial laboratory.

The declassification of fusion research by Britain, the United States and the USSR in 1958 has given research on controlled thermonuclear reactions a strong and welcome international character. Thus, although this article deals primarily with the British programme, any broad appraisal of the fusion scene must take into account contributions from many countries—not only the ones in the field initially but also others, particularly France and Germany, each of whom have built up research programmes significantly larger than that in Britain.

By the mid-1960s, this global effort had revealed many aspects of plasma behaviour. It had been shown that one condition for the production of energy in practical amounts could be achieved: plasmas could be heated to the so-called "ignition temperature", that is, the temperature at which the production of fusion power exceeds the energy losses by radiation. But a second essential condition, the confinement of plasmas for long enough times, remained unsolved and intractable.

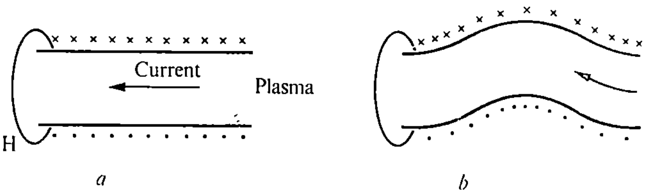

Fig. 1 Two situations in a Zeta-type apparatus. *a*, Magnetic field produces a pinching force towards the axis; *b*, a distortion in the magnetic field produces a net force which causes the distortion to grow.

The first threat to the confinement of high temperature plasma by magnetic fields came from the occurrence of "gross instabilities". Theoretical analysis supported the experimental observation that many kinds of gross instabilities existed, all of which were sufficient to prevent the desired isolation of the plasma. This is not the place to give an exhaustive list of instabilities, but it is worthwhile illustrating the problem with just one example, namely the basic problem which faced a Zeta-type apparatus. In this type of device, the gas discharge itself produces an intense magnetic field and the hope is that this field will be sufficient to isolate the plasma. Thus, in an idealized quiescent situation, the situation can be imagined as shown in Fig. 1*a*, where the magnetic field produced by and surrounding the current may be shown to produce a force $\frac{1}{c}\,\mathbf{j}\times\mathbf{H}$ towards the axis, thus "pinching" the discharge into a smaller volume.

If, however, a distortion develops in the discharge, as shown in Fig. 1*b*, the magnetic field density becomes larger on one side of the discharge than on the other and this produces a net force which causes the distortion to grow rapidly.

In other words, the discharge is unstable to simple geometrical distortions of this type and in practice, unless additional magnetic fields are introduced, the kink in the discharge grows so large that the discharge hits the wall of the container.

In the mid-1960s, not only did gross instabilities, of which this is just one example, seem possibly too difficult to control but also, even if their control could be achieved (as it was in the stellerator in the United States), the ability of magnetic fields to confine plasma seemed to be further threatened by substantial energy losses associated with the diffusion of high temperature plasma across the confining fields at a much more rapid rate than classical diffusion theory indicated. This process, known as Bohm diffusion, had displayed itself in a number of experiments and had been found to be connected with the presence of density fluctuations in the plasma. It seemed that, even if a plasma could be created inside a magnetic field geometry which was stable overall, the plasma would nevertheless not be quiescent and the remaining fluctuations in the system would stimulate diffusion and loss of plasma.

By the mid-1960s, therefore, it seemed that the degree of magnetic confinement required to sustain a useful fusion reaction might be very difficult or even impossible of attainment because of the two problems of gross magnetic-hydrodynamic instability and Bohm diffusion. Indeed, appreciation of these difficulties could be said to have led to a period of gloom in fusion research throughout the world. It was not surprising therefore that, when the Culham programme was reviewed in 1966, the outcome was a decision by the UKAEA to decrease effort on fusion research progressively to half its initial level over a period of five years, the decision being subject to alteration if developments appeared to warrant it.

Recent Progress

The research programme at Culham has retained its high quality and has remained directed towards the confinement of plasmas and, in spite of the cutback, it has therefore been able to contribute significantly to the total world attack on this problem. This world effort has produced scientific progress which has been continuous and far reaching, so much so that gross instabilities have now been understood and suppressed in many containment systems and a number of remedies for Bohm diffusion have been devised. Indeed, classical or ideal plasma containment, which is actually better than that required for a fusion reactor, has already been achieved at "low" temperatures in several machines. These machines have, however, been designed for specific purposes and the twin objectives of adequate containment time and the achievement of ignition temperature have not yet been attained simultaneously in any one machine. This world effort has increasingly put emphasis upon toroidal systems as these allow the electrical discharge to close in upon itself—that is, eat its own tail—and allow magnetic field lines to be retained entirely within the confinement vessel itself.

It is interesting to note in passing how attitudes to Zeta, the first toroidal-pinch device, have fluctuated with the current understanding of magnetic containment. At one time it was easy for the non-expert to assume that the experiment was a disappointment. In fact, with increasing insight, the operation of Zeta in a stable mode was demonstrated and the device became a most valuable source of very hot plasma and, for some years, yielded many useful data pertinent to toroidal systems. Indeed, the experiment may be regarded as the natural precursor of the Tokamak device, currently one of the most promising confinement systems.

Looking back over the past five years or so, from the British point of view, the chief milestones in scientific progress have been as follows: (1) the attainment of a detailed understanding of gross instabilities which led rapidly to their elimination by several schemes setting up magnetic fields of carefully defined geometry; (2) a growing understanding of Bohm diffusion and the demonstration of its suppression by several techniques; (3) the subsequent achievement of classical diffusion and prolonged confinement in suitably constructed devices, operating at "low" temperatures (that is, up to about a million degrees); (4) the development of laser scattering techniques which has transformed the detail and precision of experimental measurements; (5) the very successful Russian experiment on Tokamak which, together with the stabilization of the Zeta discharge, re-established toroidal discharges as credible confinement systems; (6) the evolution of sophisticated and comprehensive theoretical models to interpret and predict plasma behaviour in a wide variety of circumstances.

Present Programme

Currently, the British programme is based on four principal lines of study all aimed at resolving particular aspects of plasma containment in toroidal systems and bearing potential reactors in mind. It is unfortunately true that most experimental assemblies are difficult to describe in detail because the geometrical configuration of the magnetic field, which is set up to confine the plasma, is necessarily complex. Basically, however, in most toroidal systems the magnetic field lines follow the path that would be made by bending a helix into a circle so that the head and tail of the helix are joined up. An alternative way of picturing the magnetic field lines is to imagine a coil spring bent round into a circle to form a "doughnut" shape. The plasma is then trapped in the "doughnut-like" space inside the spring, that is, inside the magnetic field. The four systems in use at Culham set up this magnetic field in different ways. In the toroidal pinch assembly, which is the most direct successor to Zeta, the field is primarily set up by currents flowing in the discharge itself; this field is supplemented by an additional field produced by a coil wound on the toroid. The Tokamak geometry is closely related to this but with fields of different magnitude; thus the field generated by the discharge itself is dominated by the additional field produced by the external coil wound around the outside of the doughnut. In the superconducting levitron the field is produced partly by a coil wound round the outside of the doughnut and partly from a superconducting ring which is levitated within the doughnut space. In the stellerator the field is produced by a coil wound round the outside of the doughnut supplemented with a more complex winding, again on the outside of the doughnut, specially designed to produce the twisting effect analogous to a helix. These four systems are illustrated in Fig. 2*a* (the toroidal pinch and Tokamak), in Fig. 2*b* (the superconducting levitron) and Fig. 2*c* (the stellerator).

Supporting programmes are arranged to complement as effectively as possible all four chief approaches to confinement. Thus, studies of plasma production and heating are aimed at providing plasmas for experiments and exploring the techniques likely to be needed in reactors, whereas the technology programme is concerned principally with the development of superconducting coils for magnetic containment, surface physics, and the study of reactor concepts. Similarly, theoretical studies are related closely to the experimental programme with special emphasis on plasma diffusion in closed line systems and on plasma turbulence. Diagnostic work has now been concentrated chiefly on the further development of laser scattering methods, while basic studies of plasma physics have been concentrated into one area, namely non-linear plasma effects.

In recent months a great deal of public attention has been given to the prospects of producing fusion reactions from the inertial confinement of very dense plasmas generated by lasers. Culham has had a modest programme of research on dense plasmas for some years, but very large programmes of work on this subject in the United States and the USSR have recently been reported although their work is only partly declassified at present. Because resources are limited, the Culham programme will be able to keep little more than a "watching brief" on this development but, because of the ingenuity and promise of the idea and because if its topical nature, it is worth a brief description here. The basic idea is very simple: the light from a powerful pulsed laser is focused by an optical system onto a small cold pellet of deuterium. When the laser pulse hits the outside of the pellet the outside layers of deuterium are evaporated off but the inner part of the pellet is compressed to a high density. Computer calculations suggest that compressions by as much as a factor of 10^4 can be achieved and this would be sufficient to produce a sizable release of thermonuclear energy. Furthermore, the highly compressed state of matter which a successful experiment would produce is of scientific interest in its own right.

There remain, however, a number of uncertainties connected with this idea. (1) It is not clear that sufficient laser energy can be deposited rapidly in the outer layers of the pellet. (2) The process of depositing this energy may cause fast electrons to run ahead of the incoming pressure pulse, thus building up a significant back pressure. (3) Unless the geometry is strictly spherical, electric currents may produce magnetic fields which would also stop the implosion. (4) It may not be possible to produce lasers of sufficient power.

Because of these uncertainties, the magnetic confinement of plasma still seems the most promising route to thermonuclear reactors. Nevertheless, modest compression ratios have already been produced by this laser technique, and substantial improvements are in sight once more powerful lasers are available and therefore this remains an exciting area of investigation which, at the very least, will need to be kept under close observation in the future.

Future Prospects

In view of the recent scientific progress, it is now possible to say with some confidence that plasma conditions close to those needed in a reactor could be achieved in a device based upon existing knowledge of magnetic containment but suitably scaled up in size. It is therefore probable that there is no fundamental bar to producing electric power from controlled thermonuclear fusion, though it is not possible at the present time to envisage clearly the form that a practical fusion reactor would take. What does seem quite clear is that the cost of pursuing a commercial fusion reactor (whatever designs are ultimately chosen) will at least equal that involved in introducing a major fission reactor system, namely some hundreds of millions of present-day pounds sterling. Such sums are very large indeed and therefore strong incentives exist for collaboration between different countries as a means, amongst other things, of effectively sharing the cost and risks of development.

It is not possible for any project, at the stage of development now reached with fusion research, to demonstrate that the major technological programme needed to exploit the ideas being developed will be economically worthwhile ; not enough can be known about the costs involved. In the case of fusion, it is impossible to assess the cost of producing electricity, either from a fusion reactor or from whatever may be the best alternative at the relevant time, with sufficient accuracy to show that the exploitation of the fusion reactor for electricity generation will save money. The best estimates that can be made now suggest that provided reasonable technological progress is made, for example, on the production of superconducting materials, the costs of electricity from fusion and from the best fission reactor will probably be about the same provided one uses today's prices for the uranium fuel needed to feed fission reactors.

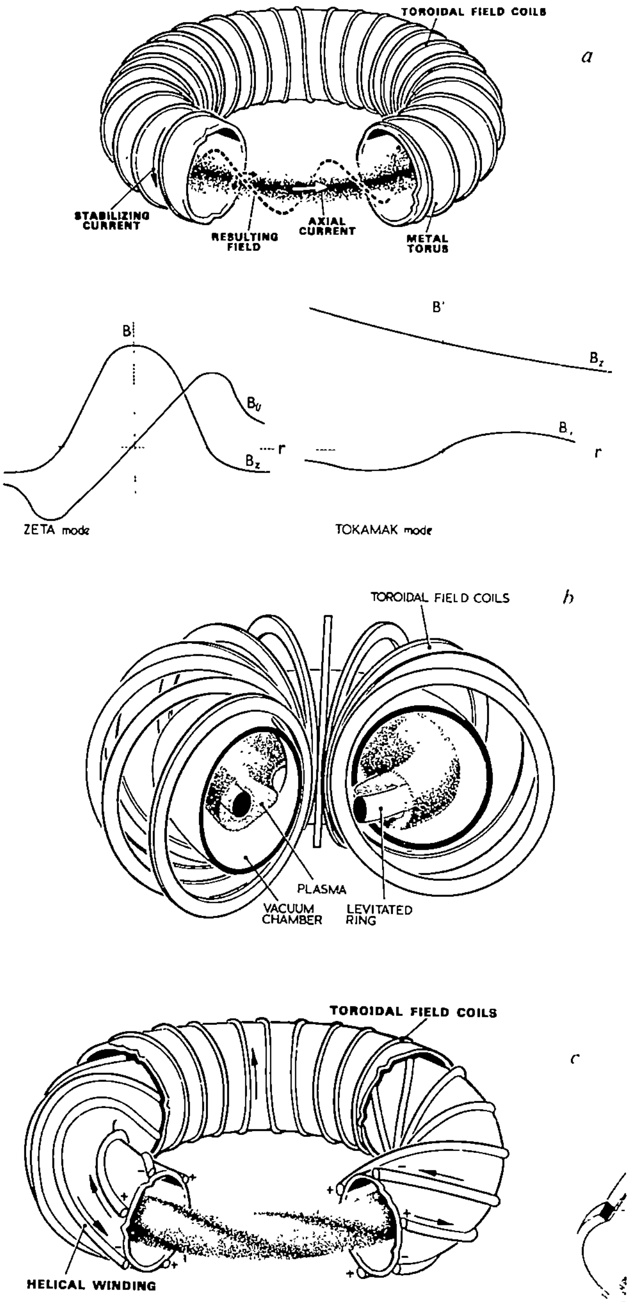

Fig. 2 Four fusion systems. *a*, Toroidal pinch and Tokamak; *b*, levitron; *c*, stellerator.

This last qualification suggests that, if uranium were in short supply and prices rose, the fusion reaction might secure an economic advantage as well as offering a means of conserving world resources of energy. The world demand for electricity shows every sign of continuing to rise while reserves of fossil fuels become harder to find and more expensive to exploit. Indeed, most developed countries expect, in the light of these two trends, to have to generate some 30–70% of their total electric power requirements from nuclear fuels by the year 2000. If this nuclear component were to be generated solely by fission in thermal reactors then a uranium shortage could develop around the turn of the century and fusion reactors would fit in as natural successors to fission reactors on this broad timescale. One actually expects, however, a substantial part of the world's nuclear generating capacity to consist by then of fast breeder reactors and these, by virtue of their highly efficient utilization of the fuel, could extend the life of low-cost uranium resources alone for at least a century and permit (with only a slight effect on the cost of power) the working of high cost reserves ; this could defer the problem of energy sources for a very long time indeed. Indeed by extracting uranium from seawater—a feasible but expensive operation—the world's demands for energy could be met indefinitely by fast breeder reactors.

Even though no economic advantage from fusion reactors can now be proved, it should be noted that with the expected large increase in the demand for electricity, which can be met most economically by nuclear power, even a very small differential advantage for fusion could mean large savings arising from its exploitation. Moreover, although both fission and fusion reactors produce radioactivity, the amount of radioactivity is much less, and the potential problems are much easier to guard against, for fusion reactors than with any of the current fission reactors. This will give the fusion reactor environmental and consequently economic advantages.

Two contradictory conclusions could be drawn from this. First, that the economic advantages of fusion are uncertain and that the so-called "energy crisis" will be postponed for a very long time by the fast breeder reactor and that in consequence no work on controlled fusion should or need be done. Or, second, that it is now known that fusion reactors can be built, that with the immense effort now being devoted to them especially in the United States and the USSR, they almost certainly will be built (whether immediately economic or not) and that, although not enough is known to tell how much they will cost, they may possibly offer the option to enjoy cheaper and cleaner power than ever before.

Clearly difficult decisions will arise when the time comes for a major technological programme leading to commercial size fusion reactors. Work in Britain at present is, however, primarily scientific in character and modest in expenditure compared to that which eventually will be needed in a major "scaled up" programme. Looking to the future, it seems clear that a purely national research programme would be lost against the magnitude of the task. On the other hand, a European programme, if pursued in concert, would be likely to achieve technical success and could well produce commercial success at modest cost to each participating country. It would provide the best means of ensuring that Britain and Europe jointly and severally were able, when the time came, to occupy their proper place in the world market for fusion power.

Programme Review

The year 1972 marked the end of the rundown in the Culham programme decided upon five years previously by the UKAEA. To assist the authority in deciding what the future programme should be, a panel of experienced scientific managers and administrators from industry, the Science Research Council, and universities, as well as from within the authority, under the chairmanship of Sir Harrie Massey, was asked to review the British fusion research programme and to make recommendations as to its broad future. The views of the panel were accepted by the authority, and the minister, Mr Boardman, has recently announced his acceptance of their recommendations which were broadly along the lines indicated in this article.

The minister made it clear that the government's decisions depended on full collaboration with Europe. Here, also, good progress is being made. The Euratom programme on fusion is very successful and Britain's accession to the EEC makes it natural for it to join in that programme as a full partner. The Council of Ministers of the community have indeed already agreed in principle that there should be a Contract of Association between the authority and the community in respect of the Culham fusion programme. The exact content of the future Culham programme will therefore be a matter for discussion with the appropriate Euratom boards. It is possible, however, to see the likely next steps in a Euratom programme. There is now a general feeling of confidence among fusion experts that plasma conditions approaching those needed in a reactor may be achieved in a device using what is already known about magnetic confinement but suitably scaled up in size. Such a machine with a capital cost of, say, £7 million would be considerably larger than those currently in use or under construction at Culham and would constitute the logical next step towards a fusion reactor. This logic has already been accepted by the United States, the USSR, and West Germany who propose to construct devices of this size and bring them into operation in the next three or four years. Each of these machines will, when built, represent a somewhat different approach to a reactor, and several more of similar size will be needed to explore this next stage. It seems likely that, when decisions are made within Euratom, Culham will be seen as a likely site for one of these larger machines and this will in its turn determine the main features of Culham's future programme.

The acceptance of the recommendations of the panel under Sir Harrie Massey, together with the prospective Contract of Association, imply a halt to the rundown of the fusion research programme at Culham and, indeed, over the next few years there will be a modest expansion of the work up to a point roughly halfway between the maximum size of the programme in 1966 and the minimum size reached in 1972. It seems fair to interpret this decision as a compliment, first to the scientific excellence of the world-wide work on fusion and second to the strenuous efforts made by Dr Pease and his colleagues at Culham to maintain a powerful and well-orientated effort in the face of a significant cutback of the programme over the past few years.

Increasing Involvement

Looking to the future, as fusion research gets closer to the point of commercial exploitation a European programme will need to involve industry more and more and it will be necessary to form international organizations involving governments, research and development authorities, industry and users. Such organizations are neither easy to form nor easy to manage. For the next few years, however, it seems sufficient to concentrate on welding together a good scientific team by building up on the existing and successful Euratom coordinated effort. The really difficult decisions on producing power from controlled fusion, which are very much larger and very much more complex than those that have to be made at present, will need to be faced in the future.

Possibility of Natural Diamond Synthesis under Conditions of Cavitation, occurring in a Fast-moving Magmatic Melt

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The formation of diamonds in nature can be explained by cavitation occurring in magma flowing in kimberlite pipes.

THE greatest difficulty in understanding the origin of natural diamonds is to discover a geological situation in which pressures above 70 to 100 kbar and temperatures above 1,200° C are reached. In some cases diamonds have been seed grown under low pressure ($P=1$ torr). But this method is feasible only when building up a crystal lattice, and not when synthesizing it anew[1]. Moreover, a marked fractionation of carbon isotopes[2] accompanying the formation of such diamonds would result in an isotopic composition of diamonds which is quite untypical for natural conditions. The mineralogical composition of the diamond bearing rock kimberlite indicates that it was never subjected to pressures above 20 to 25 kbar: it contains pyrope (synthesis pressure 10 to 20 kbar), but not minerals formed under pressures of 35 to 90 kbar such as coecite, stishovite (dense modifications of SiO_2), and piezolite (artificial alumosilicate, synthesized under $P=45$ kbar and $T=700°$ C), whose formation must precede that of diamonds. Here I draw attention to the existence of a hydrodynamic phenomenon, cavitation, in which high pressures and temperatures occur at several points, whereas the ambient parameters are, on average, moderate. Cavitation occurs in fluids containing dissolved gases, and high gas contents are distinctive features of diamond-bearing kimberlite magma, saturated with carbon dioxide, whose relics invariably occur in kimberlites as dispersed carbonates.

Under a certain initial pressure P_0 the flow is a single phase fluid-gas. As the pressure drops below a critical value, perhaps as a result of increased flow rate, incipient bubbles of gas come into existence. Now, if the pressure rises again before the gas bubbles have associated into a continuous phase, the bubbles will collapse. In these conditions the pressures at the points towards which the bubbles collapse are greater, by several orders, than the initial ambient pressure. Simultaneously, because of adiabatic compression, the temperature will increase. Such cavitation is known to cause erosion and destruction of propeller blades, as well as components of pumps and pipings.

Kimberlite pipes (diatremes) typically extend vertically with a length of the order of several kilometres and diameter of the order of tens of metres. Under such circumstances cavitation inception is associated with magma flowing quickly in a channel of variable cross section.

According to Bernoulli's relation, pressure across contracted portions of a channel will decrease as

$$P_0-P=\frac{\rho}{2}\bar{u}^2\left[\left(\frac{D}{d}\right)^4-1\right] \qquad (1)$$

where D = mean diameter of channel; d = diameter of contraction; $\bar{u}$ = average rate of magma through channel.

The initial pressure P_0 in the magma should meet the requirements of synthesis for the most barophilous minerals of kimberlite rocks, whose formation excludes the cavitation synthesis mechanism; these include pyrope. Because pyrope is synthesized at pressures approaching 20 kbar, this value should be regarded as the required original pressure.

The average velocity u is controlled by hydraulic losses in the magmatic channel; with $D=n\times 10$ m, u is about $n\times 100$ m s^{-1}. The pressure under which carbon dioxide fully dissolves in magma at approximately 1,000° C is ~11 kbar. Thus, it will be necessary to reduce the channel diameter no more than 2 to 3 times to produce the pressure required to cause cavitation in a magmatic melt.

The question whether the high pressures and temperatures necessary for the synthesis of diamonds do occur in the process of gas bubble collapse can be answered by calculating the collapsing conditions.

Introducing simplifying assumptions (ideal character of compressed gas, negligence of dissipative processes, and compressibility and viscosity of fluid) the process of gas bubble collapse can be expressed as follows[3]:

$$\left(\frac{dR}{dt}\right)^2=\frac{2P}{3\rho}\left[\left(\frac{R_0}{R}\right)^3-1\right]-\frac{Q\left[\left(\frac{R_0}{R}\right)^3-\left(\frac{R_0}{R}\right)^{3\gamma}\right]}{1-\gamma} \qquad (2)$$

where P = external pressure (which can be assumed unchanged and equal to P_0); ρ = fluid density; R_0 = maximum radius of bubble at the initial moment of collapse; Q = gas pressure in bubble at $R=R_0$; γ = adiabatic index.

The maximum pressure in the matter being compressed by the closing walls of a bubble can be deduced from the relation

$$P_{max}=Q(R_0/R_{min})^{3\gamma} \qquad (3)$$

where R_{min} = minimum size of a bubble reached during collapse.

The maximum size of a bubble is dependent on the magnitude of rarefication ΔP at the moment of gas bubble growth, the fluid density ρ and the bubble growth time t_g, and is defined by the relation

$$R_0=(2\Delta P/3\rho)^{1/2}\,t_g \qquad (4)$$

The time of growth can be defined as the period during which the fluid passes the channel contraction zone.

An approximate calculation shows that at the moment a gas bubble collapses in a medium with original pressure near 20 kbar ($R_0=20$ cm, $R_{min}=0.5$ cm), the matter is compressed at a pressure of n 1,000 kbar, substantially greater than that obtainable artificially or conceivable in any other natural processes. The matter of a gas-filled bubble, when quickly compressed, can be adiabatically heated to temperatures much greater than the initial T_0:

$$T_{max}=T_0(R_0/R_{min})^{3\gamma-3} \qquad (5)$$

The adiabatic increase of temperature in the points of bubble collapse removes the necessity of a high initial temperature of the ambient medium and permits a T_0 of 1,000–1,100° C at which a newly formed diamond, after dissipation of the pressure in the zone of compression, will be in conditions corresponding to metastability. The temperature of magma has been considered to be at least 1,500° C on the basis of the PT conditions of diamond synthesis. But in the absence of a special medium[4], this temperature should be at least 2,000 K. Apart from the difficulties in accounting for a transition of diamond into a metastable state, this is inconsistent with the presence of minerals associated with the diamond and having a relatively low melting temperature; also contact phenomena are poorly expressed (absence of fused walls in the magmatic channel and absence of xenolites—debris of intrusive rocks, carried by magma).

There is also the lower temperature boundary, limited by magma viscosity. Investigation of the collapse of a spherical space, taking due account of the fluid viscosity[5], shows that the dynamics of the process in this case depends on the Reynolds number:

$$R_e = \frac{R_0}{\nu}\sqrt{\frac{P_0}{\rho}} \qquad (6)$$

where ν = kinematic viscosity. Viscosity impedes fluid acceleration during collapsing. At $T = 800°$ C the viscosity has such a pronounced effect that for plausible initial radii the Reynolds numbers fall below the critical values (the critical value of the Reynolds number $R_{e_1} = 8.4$).

Thus, the optimum temperature of a melt clearly approaches 1,000 to 1,100° C. It is just at this initial temperature and at the original pressure, $P \simeq 20$ kbar, that the conditions occurring in the zone of compression of cavitation bubbles conform to a diamond-stable domain.

This does not solve the entire problem of synthesis, because the kinetics and relaxation conditions are also important. The process of diamond synthesis does proceed sufficiently quickly. This is proved by the production of synthetic diamonds by explosion, in which high pressures (300 to 500 kbar) are maintained for not longer than $(6-10) \times 10^{-6}$ s (ref. 6). The time of bubble collapse is found by solving equation (1), which at $\gamma = 4/3$ gives the relation

$$t_{\text{coll}} = R_{\min}\sqrt{\frac{2\rho(\xi-1)}{P_{\max}}}\left[1 + 2/3\left(\xi-1\right) + \frac{1}{5}\left(\xi-1\right)^2\right] \qquad (7)$$

where $\xi = R_0/R_{\min}$. Substituting numerical quantities of appropriate values, $t_{\text{coll}} = 2 \times 10^{-3}$ s. This exceeds by almost three orders of magnitude the time required for diamond synthesis in explosions.

In addition the rate of bubble wall convergence may, over a certain element of convergency radius, exceed the speed of sound in the medium. As a result, a spherically converging shock wave may break off the bubble surface. The occurrence of high pressures in the vicinity of the shock wave focus must result in the inception of a diamond seed at the centre of the bubble. The lattice of this diamond seed will govern the crystallization of the main body of the diamond under the compressing effects of the bubble converging walls.

The problem of relaxation lies in the necessity to provide a very rapid transition of synthesized diamonds into a metastable state. After the pressure drops to 1 atm, the diamond remains stable at temperatures less than 1,000° C; at a pressure of 20 kbar the diamond can remain stable up to 2,000° C (ref. 7).

If temperatures remain above these limits after the pressures reduce, the diamond will leave the boundaries of stability and transform into graphite. Dissipation of heat, following the synthesis process, is always slower than the pressure decrease. Therefore, graphitization seems to be inevitable. But even assuming that diamond somehow cools down rather quickly, mechanical stresses, arising in a crystal because of a temperature gradient, will result in cracking and destruction of the diamond, this being the quicker the larger is the crystal. Nevertheless, large and perfect crystals do occur naturally.

This can be explained by examining the cavitation effect. At superhigh pressures, built up during the collapse of a gas bubble, the diamond compression is found to be sufficient for thermodynamic self-cooling to take place as a result of adiabatic expansion which accompanies the super high pressures. Adiabatic cooling is possible only when an expanding body does certain work against pressure forces, acting from outside. This condition is not satisfied, for example, in technical devices of static compression, in which the pressure is released from outside; but it is brought about during cavitation synthesis, because here the process of relaxation is accompanied by the inception of an inverse shock wave, which is formed because of the resilient expansion of compressed matter.

The temperature decrease can easily be evaluated. Entropy variation as a total differential at parameters T and P is given by

$$\mathrm{d}S = \left(\frac{\partial S}{\partial T}\right)_P \mathrm{d}T + \left(\frac{\partial S}{\partial P}\right)_T \mathrm{d}P$$

From the adiabatic process relation, $\mathrm{d}S = 0$. Taking into account the well-known correlations of thermodynamics,

$$\left(\frac{\partial S}{\partial T}\right)_P = \frac{C_p}{T} \text{ and } \left(\frac{\partial S}{\partial P}\right)_T = -\left(\frac{\partial V}{\partial T}\right)_P$$

and bearing in mind that $\frac{1}{V}\left(\frac{\partial V}{\partial T}\right)_P = \alpha$

where α = thermal expansion coefficient,

$$(C_p/T)\,\mathrm{d}T = \alpha V \mathrm{d}P$$

Integrating and assuming C_p is independent of temperature

$$\ln\frac{T_{\max}}{T_0} = \frac{\alpha_{T_0} V}{C_{P_{\max}}}(P_{\max} - P_0) \qquad (8)$$

Assuming $\alpha = n \times 10^{-5}$ deg^{-1}, $V = 3.43$ cm^3 mol^{-1}, $C_p =$ 6 kcalorie deg^{-1} mol^{-1}

$$\ln\frac{T_{\max}}{T_0} \sim 10^{-6} P_{\max} \qquad (9)$$

Thus, at pressures $P_{\max} = 100$ kbar the adiabatic decrease of temperature is only a few degrees, negligibly small for the ultimate pressures in technical devices, as well as for the ultimate pressures in any conceivable geotectonic process. But, as shown earlier, pressures in excess of 1,000 kbar are encountered in cavitation. In this case the temperature may decrease adiabatically 3 to 10 or more times. Thus, at the peak pressure and synthesis, 2,000 kbar, the temperature in the relaxation process will decrease by 1,000 K, even if the maximum temperature is 10,000 K.

Two unique situations are at once created. First, the diamond momentarily attains a metastable state—it passes the entire process of relaxation in the domain of its thermodynamic stability, completely avoiding graphitization. Second, because of a momentary decrease in temperature, no mechanical stresses occur because cooling is effected through losses of internal energy and not by heat transfer.

Many problems concerning the origin of diamonds can thus be explained in terms of cavitation.

The suggested mechanism of diamond synthesis not only shows how the required pressures and temperatures occur under natural conditions, but also explains why diamonds are found imbedded into rocks which are free of any traces of those very high pressures and temperatures necessary for diamond formation.

Because in the course of cavitation synthesis the inception of each crystal proceeds individually, there are no limitations involving the total concentration of carbon in the medium. These limitations arise from the idea that diamonds crystallize out of a supersaturated solution, this being feasible only at a sufficiently high concentration of carbon in a melt ($\simeq 30\%$, ref. 8). That has been one of the most difficult problems of diamond origin because carbon never occurs naturally in such high concentrations.

Many natural diamonds have peculiar internal morphologies indicative of a series of interruptions during their synthesis. This is in accordance with the pulsatory movement of gas bubbles caused by compression[9]. Moreover the newly formed diamond surrounded by a gaseous mantle is the most likely nucleus for cavitation when suitable conditions arise again.

These ideas explain the occurrence of neighbouring empty and diamond-bearing pipes; the crystallographic specificity of diamonds in individual pipes; the presence of diamonds in kimberlite rock as individual crystals; the occurrence of diamonds exactly in kimberlite, which is a unique kind of CO_2-saturated fast moving magma; the similar carbon isotopic composition of diamond and endogenetic carbon dioxide; the paragenesis of pyrope and diamond; and the phenomenon of the split of diamonds and separate grains of other minerals while bodies such as xenolites remain untouched.

Finally, why have large crystallographically perfect diamonds, such as those occurring naturally, never been made artificially, in spite of the thorough selection of reactive systems, accurate control of the process and application of amply high temperatures and pressures? Evidently, because the cavitation technique used *in vivo* has never yet been tried in the laboratory.

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The Pliocene Time Scale: Calibration of Planktonic Foraminiferal and Calcareous Nannoplankton Zones

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The Pliocene spans the interval between 5 and 1.8 m.y.; its lower and upper limits can be determined by multiple micropalaeontological criteria. Within the Pliocene twelve planktonic foraminiferal and seven calcareous nannoplankton datum levels can be calibrated to the palaeomagnetic time scale.

Estimates on the time span of the Pliocene have varied widely, from a little over 1 m.y.[1–5] to over 10 m.y.[6,7]. The age of the Miocene/Pliocene boundary itself has been estimated to be as young as 2.7–3.0 m.y.[1–4] and as old as 15 m.y.[8]. Estimates by most vertebrate palaeontologists have ranged between 10–12 m.y. because of the supposed initial appearance of the three-toed *Hipparion* in the lower part of the stratotype Pontian of the eastern Mediterranean, which was thought to coincide with the Miocene/Pliocene boundary[9,10]. More recent estimates of 7 m.y. for the Mt Capanne granodiorite on Elba[11], and 6.6–8.0 m.y. for the volcanic tuffs intercalated with Messinian beds in Morocco[12], have indicated that the Miocene/Pliocene boundary, as defined in the marine sedimentary succession in the Mediterranean region, is younger than 7 m.y. The date of 4.7 m.y. on the Orciatico trachyte which metamorphoses lower Pliocene clays in Tuscany[13] and integrated biostratigraphic palaeomagnetic studies on deep-sea cores[14] have indicated that the boundary is older than 4.5 m.y. and an age of 5 m.y. has been suggested[15,16]. Recent investigations have corroborated this estimate[17–19].

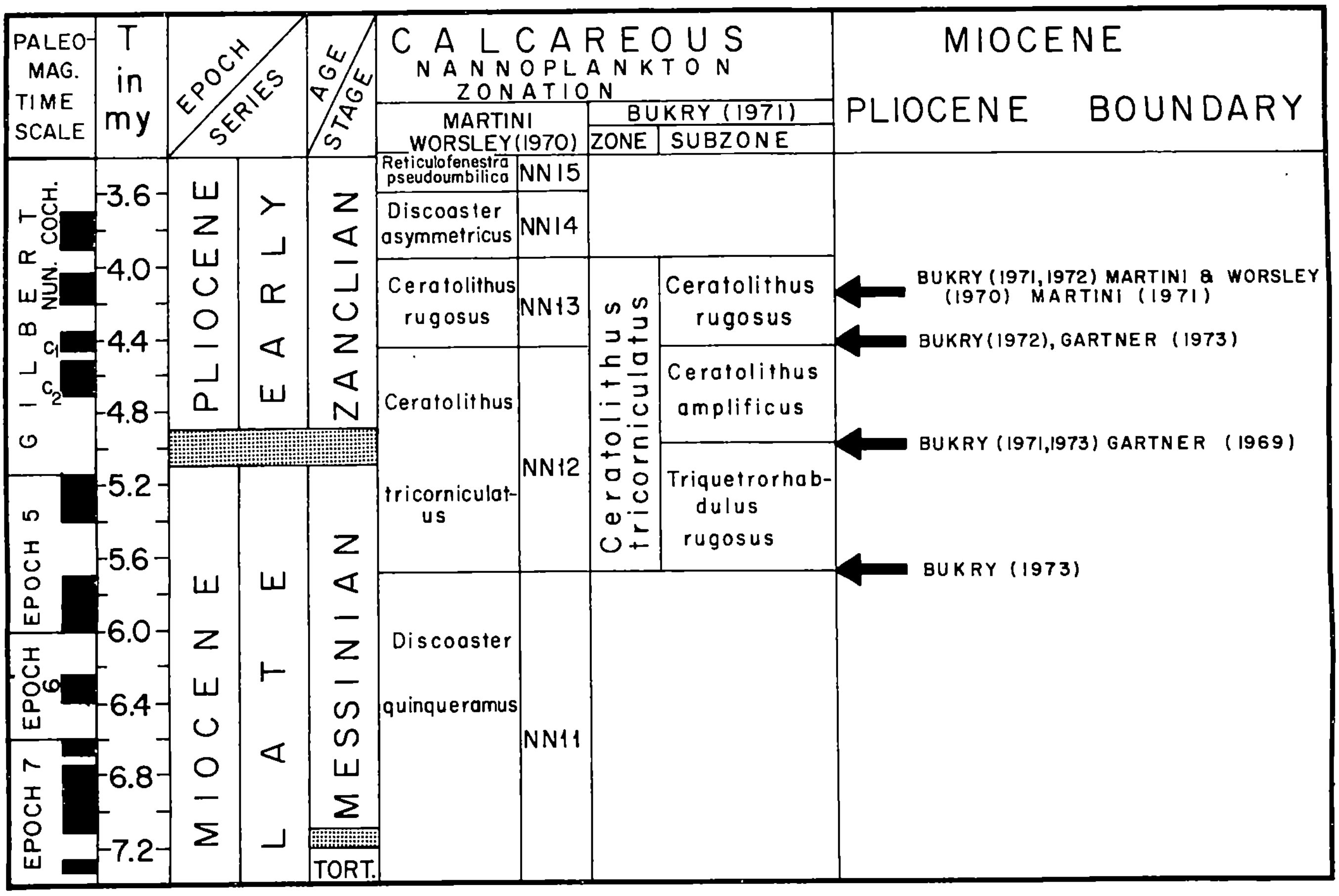
Similarly, age estimates of the Pliocene/Pleistocene boundary have varied over a wide range from about 0.6 m.y.[20,21] (Emiliani qualified these estimates with a caution that correlation with the base of the stratotype Calabrian in Italy had not been achieved) to over 4 m.y. based on the dates of 3.4 (ref. 22) to 4.3 (ref. 23) m.y. on the earliest Villafranchian faunas which are attributed by many vertebrate palaeontologists in Europe and the Soviet Union to the Pleistocene. Recent data have shown that the continental type Villafranchian is essentially late Pliocene (pre-Calabrian), that the Villafranchian in a wide sense is as young as 1.0 m.y.[24], and that the base of the stratotype Calabrian at Santa Maria di Catanzaro, that is, the Pliocene/Pleistocene boundary, is about 1.8 m.y. old[25–27].

I present here an estimate on the age of the Miocene/Pliocene boundary based on considerably firmer data than those available up to now[28–32], suggest a new planktonic foraminiferal zonation of the Pliocene, correlate these zones with the Pliocene calcareous nannofossil zones and calibrate them both to the palaeomagnetic time scale.

Age of the Miocene/Pliocene Boundary

The development of a discrete supplementary aperture on the spiral side of the test has been suggested as the distinguishing criterion separating the planktonic foraminiferal genus *Sphaeroidinella* from its ancestor *Sphaeroidinellopsis*[33]. The initial evolutionary appearance of *Sphaeroidinella dehiscens* (including *forma immatura*) from its immediate ancestor *Sphaeroidinellopsis subdehiscens paenedehiscens* was said to denote the base of Zone N19 (ref. 34). The initial appearance of *S. dehiscens* was recorded about 40 feet above the base of the Zanclian stage in Sicily[35] so that the Miocene/Pliocene boundary was said to lie within the subjacent Zone N18. The base of Zone N18 is placed at the bottom of the first evolutionary occurrence of *Globorotalia tumida* from its immediate ancestor, *G. plesiotumida*[36].

Table 1 Placement of Miocene/Pliocene Boundary by Calcareous Nannoplankton Biostratigraphers



The *S. dehiscens* Datum, as originally defined by Bandy[37,38], refers to the initial appearance of large, robust individuals of *S. dehiscens* with a broad, scalloped, everted flange. This level is associated with the extinction of the *Sphaeroidinellopsis* group, is slightly older than the extinction level of *G. multicamerata* and *Globoquadrina altispira*, is coincidental with the planktonic foraminiferal N20/N21 zonal boundary and has been shown[39] to be about 3 m.y. old on the palaeomagnetic time scale. It does not correspond to the Miocene/Pliocene boundary as defined in the marine sedimentary succession in the Mediterranean.

Thus we see that the definition of the *S. dehiscens* Datum by both Bandy[37,38] and Blow[34] is clearly and unequivocally stated but refers to biostratigraphic events which are significantly different in space and time. The *S. dehiscens* Datum of Bandy[37,38] is about 3 m.y. old[14]; that of Blow[34] about 4.8 m.y. old[19] (see also Tables 2, 3 and Fig. 1).

In terms of the calcareous nannofossils the Miocene/Pliocene boundary has been variously placed at the base of the *Ceratolithus tricorniculatus* Zone (NN12)[40], within the *C. tricorniculatus* Zone[41–43], at the *C. tricorniculatus*/*C. rugosus* (NN12/NN13) boundary[44,45], or within the *C. rugosus* Zone (NN13)[46–49] (Table 1). In a recent investigation[50] the Miocene/Pliocene boundary is correlated with the *C. rugosus* Datum (base Zone NN13). This was based on an earlier study[51] showing that the *C. rugosus* Datum occurred in the southwest Pacific core CAP 38 BP at a level (625 cm) dated within Zone N18 on planktonic foraminifera[52].

S. dehiscens has been shown[14] to range well below the age of 4.2 and 4.1 m.y. estimated for the two levels determined by Parker[52] and Blow[53], respectively, in core LSDH 78 P for the

Table 2 Pliocene Planktonic Foraminiferal Datum Levels

Time (m.y.)		Datum level	First occurrence (FO) Last occurrence (LO)
~1.8	III	*Globigerinoides fistulosus*	(Top Olduvai) LO
~1.95	IV	*Globorotalia truncatulinoides*	(Base Olduvai) FO
~2.95	V	*Sphaeroidinellopsis* spp.	LO
~3.34	VI	*Globorotalia margaritae*	LO
~3.6	VII	*Pulleniatina* (primarily *P. primalis*)	Sinistral to dextral coiling change
~3.7	VIII	*Globigerina nepenthes*	LO
~4.3	IX	*Pulleniatina spectabilis*	FO
~4.8	X	*Sphaeroidinella dehiscens*	FO
~4.9	XI	*Globorotalia tumida*	FO
~5.0	XII	*Globoquadrina dehiscens*	LO

Data from refs. 14, 19 and this paper.

Table 3 Estimated Age of Biostratigraphically Important Pliocene Datum Levels in Core 6, Hole IIIA (Orphan Knoll)

Section	Depth (cm)	Estimated age (m.y.)	Calcareous nannoplankton datum	Planktonic foraminiferal datum
2	134	2.9	—	*Globoquadrina altispira* and *Sphaeroidinellopsis* spp. (E)
	140	3.05	*Reticulofenestra pseudoumbilica* (E)	—
3	10	3.48	*Ceratolithus tricorniculatus* (E)	—
	18	3.7	—	*Globigerina nepenthes* (E)
	55	4.45	*Ceratolithus rugosus* (FO)	—
	70	4.8	—	*Sphaeroidinella dehiscens* (FO)
	75	4.9	—	*Globorotalia tumida* (FO)
	80	5.0	*Ceratolithus amplificus* (FO)	*Globoquadrina dehiscens* (E)

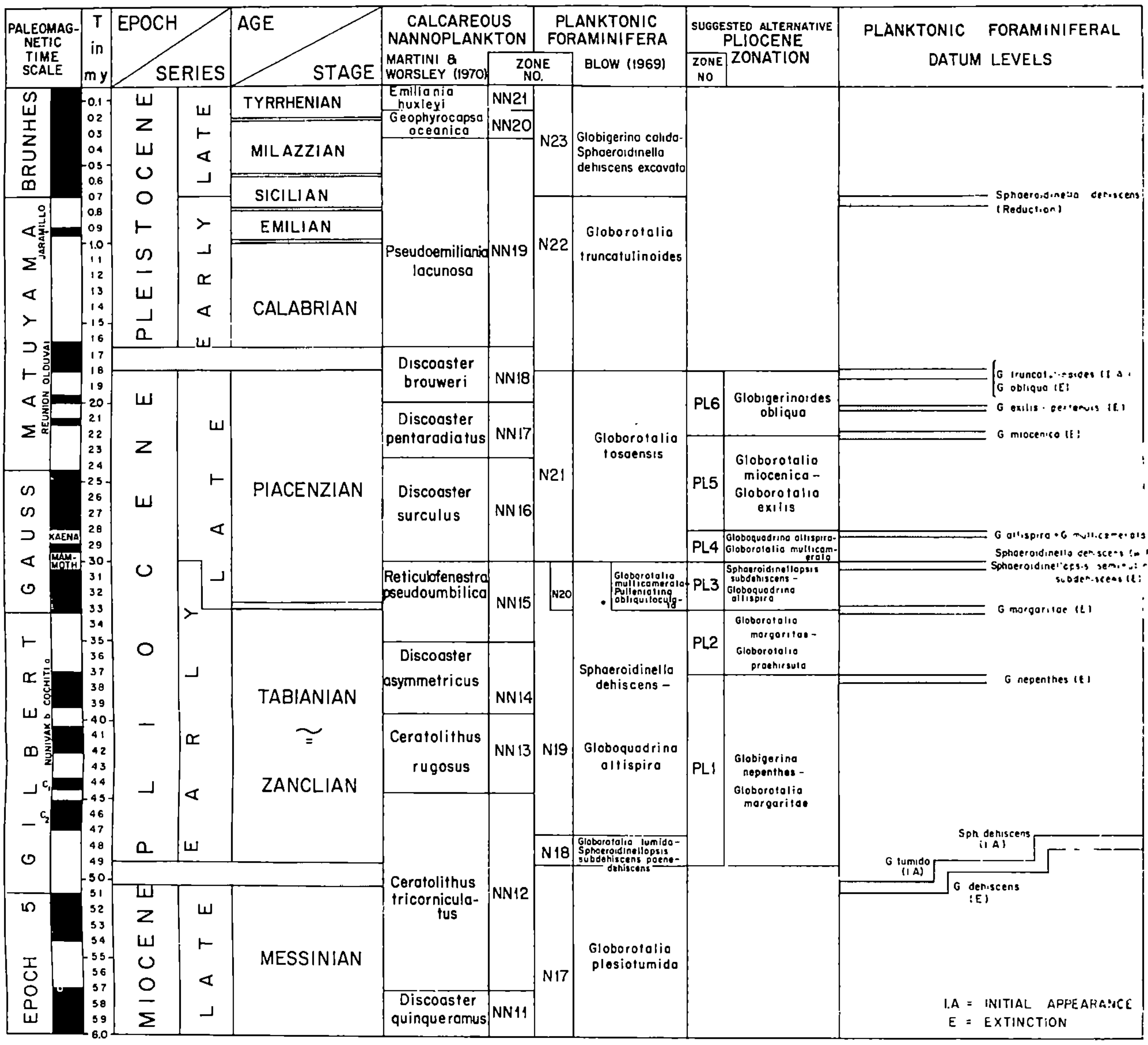


Fig. 1 Correlation and calibration of Pliocene planktonic foraminiferal and calcareous nannoplankton zones and datum levels.

N18/N19 boundary and of 4.3 m.y. for the base of Zone N18 (=Miocene/Pliocene boundary) by extrapolation in that core[14]. In fact it was shown[14] that the Miocene/Pliocene boundary must be older than 4.5 m.y., the age of the bottom of core V24–59 in which *S. dehiscens* was present. The initial appearance of *C. rugosus* is recorded within the Gilbert "C" in cores V24–59 and RC12–66 at about 4.45 m.y.[50]. Thus, the Miocene/Pliocene boundary, as determined by planktonic foraminiferal criteria on core LSDH 78 P (refs. 52, 53) and on CAP 38 BP (ref. 52), is younger than the true Miocene/Pliocene using the criteria originally suggested for recognizing this boundary. The *S. dehiscens* Datum (base Zone N19) and the *G. tumida* Datum (base Zone N18) are dated in this paper at 4.8 and 4.9 m.y. respectively and this is in perfect agreement with independent estimates on these levels[19]. I conclude that the estimate of the Miocene/Pliocene boundary at the base of the *C. rugosus* Zone (~4.5 m.y.)[50] is too young and that this level lies within the lower Pliocene.

It should be pointed out that the recognition of the *S. dehiscens* Datum can be extremely difficult; thus, if the first appearance of *S. dehiscens* is not accurately determined miscorrelation by as much as a whole nannofossil zone may result. For this reason the first appearance of *G. tumida* and the extinction of *Globoquadrina dehiscens* (~5.0 m.y.) are here considered more reliable indicators of the Miocene/Pliocene boundary. The last occurrence of *Globoquadrina dehiscens* was recorded at 674–676 cm in CAP 38 BP (ref. 52), a little over 0.5 m below the initial appearance of *C. rugosus* at 625 cm (ref. 51).

The Miocene/Pliocene boundary has been correlated with the first appearance of *C. amplificus*[42], a species intermediate between *C. tricorniculatus* and *C. rugosus*. *C. amplificus* appears at a level (18 m) dated at about 4.9 m.y. in core RC12–66 (ref. 19) and is coincidental with the base of Zone N18 in that core.

The co-occurrence of *C. tricorniculatus* and *C. amplificus* is restricted to the lower part of the lower Pliocene (Zanclian) in Sicily[50]. *C. amplificus* initially appears approximately 6 m (but does not become abundant until about 18 m) above the base of the Zanclian (S. Gartner, personal communication) at the newly proposed type section at Capo Rosello[54]. If this level corresponds to the level dated at about 4.9 m.y. in core RC12–66, which is approximately equivalent to the base of Zone N18, then the Miocene/Pliocene boundary may be somewhat older than this level and an estimate of 5.0 m.y. is suggested here. The initial appearance of *S. dehiscens* was recorded[35] about 40 feet above the base of the Zanclian so that the Miocene/Pliocene boundary is somewhat older than the N18/N19 boundary. As noted below, it actually lies at the base of Zone N18 or even slightly lower; for all practical purposes it may be drawn at the base of Zone N18. Thus the Miocene/Pliocene boundary lies within the stratigraphic range of *C. tricorniculatus* (NN12) and in a recent subdivision of the *C. tricorniculatus* Zone into three subzones (from the bottom), *Triquetrorhabdulus rugosus*, *C. amplificus* and *C. rugosus*, the Miocene/Pliocene boundary was drawn at the base of the *C. amplificus* Subzone[42].

In summary, it seems that a set of multiple criteria have been developed for determining the Miocene/Pliocene boundary in a way similar to that suggested for the Pliocene/Pleistocene boundary[55]. These criteria include: planktonic foraminifera —extinction of *Globoquadrina dehiscens*, first evolutionary appearance of *S. dehiscens* and *G. tumida*; calcareous nannoplankton—first evolutionary appearance of *C. amplificus*. These multiple criteria occur within the lower part of the Gilbert Reversed Epoch on the palaeomagnetic time scale. The Gilbert/Epoch 5 boundary has been dated at about 5.2 m.y. (A date of 5.1 m.y. was estimated for the top of Magnetic Epoch 5 by Foster and Opdyke[56] and revised by Talwani *et al.*[57] to 5.18 m.y.). For practical purposes the Miocene/Pliocene boundary may be said to occur within the interval of 4.9–5.1 m.y.

Correlation and Calibration of Pliocene Calcareous Planktonic Zones

The basic parameters within which the Pliocene events are calibrated are the Pliocene/Pleistocene boundary (~1.8 m.y.) and the Miocene/Pliocene boundary (~5.0 m.y.). The former is characterized by several biostratigraphical events which, taken together, approximate the upper boundary of the Pliocene[27]. They include: extinction of *Globigerinoides obliqua extrema* at the base of the Olduvai Event, about 1.8 m.y.; initial appearance of *G. truncatulinoides* somewhat higher, within the lower part of the Olduvai Event, about 1.75 m.y.; extinction of discoasters near the top of the Olduvai Event, about 1.65 m.y.; extinction of *Globigerinoides fistulosa* slightly above the top of the Olduvai, about 1.60 m.y. The Pliocene/Pleistocene boundary is drawn here, arbitrarily, at the base of the Olduvai Event, at about 1.8 m.y. The Miocene/Pliocene boundary is characterized in a similar manner by several biostratigraphical events which, together, approximate the lower boundary of the Pliocene. These include the extinction of *Globoquadrina dehiscens* at about 5.0 m.y.; first evolutionary appearance of *G. tumida* and *C. amplificus*, at about 4.9 m.y.; first evolutionary occurrence of *S. dehiscens*, at about 4.8 m.y. The Miocene/Pliocene boundary is drawn here at 5 m.y.

Nine planktonic foraminiferal datum levels have recently been calibrated to the palaeomagnetic time scale for the past 4 m.y.[14]. Seven of these (numbers IX–III) are of Pliocene age. An additional three (XII–X) are suggested here (Table 2) based on more recent data obtained in my laboratory (Table 3) and elsewhere[19]. Eleven distinctive calcareous nannofossil datum levels have also been calibrated with the palaeomagnetic time scale[50]. Those of particular importance to this discussion of Pliocene chronostratigraphy are shown in Table 4.

In Fig. 1 the Pliocene calcareous nannoplankton and planktonic foraminiferal zones are placed in a time framework. A summary of the basis for this correlation is presented below.

Table 4 Late Miocene/Pliocene Calcareous Nannoplankton Datum Levels

Time (m.y.)	Datum level	First occurrence (FO) Last occurrence (LO)
~1.8	*Discoaster brouweri*	LO
~2.1	*Discoaster surculus*	LO
~2.4	*Reticulofenestra pseudoumbilica*	LO
~3.6	*Ceratolithus tricorniculatus*	LO
~3.95	*Discoaster asymmetricus*	FO
~4.45	*Ceratolithus rugosus*	FO
~4.9	*Ceratolithus amplificus*	FO
~5.7	*Discoaster quinqueramus*	LO

Data from refs 50 and 51.

A continuous, condensed carbonate sequence spanning the interval of latest Miocene to early late Pliocene (>5– <2.8 m.y.) was cored at Site 111 (Orphan Knoll) in the Labrador Sea during Leg 12 of the Deep Sea Drilling Project[58]. Calcareous nannoplankton zones NN12 to NN16 were recorded, each zone having an average thickness of 10–20 cm (ref. 59). Various planktonic foraminiferal datum levels were also observed[60] and using the extinction level of *Sphaeroidinellopsis* spp. (2.9 m.y.), *Globigerina nepenthes* (3.7 m.y.) and *Globoquadrina dehiscens* (5.0 m.y.) an estimation of age against depth was made for the interval of 2.8 to 5 m.y.

A re-examination of the planktonic foraminifera and calcareous nannoplankton from the critical intervals in Hole 111A has been made. Below 80 cm the sediment shows considerable dissolution and is too strongly condensed for biostratigraphical evaluation. The initial appearances and extinctions of several important taxa and their calibration with the palaeomagnetic time scale are shown in Table 3.

Data from DSDP Leg 13 in the Mediterranean Sea are also pertinent to this discussion. The *Sphaeroidinellopsis* Acme Zone occurs in the basal part of the early Pliocene transgression and was said[61] to correspond to the lowermost part of the *C. tricorniculatus* (NN12) Zone, which spans the interval between 5.7–4.45 m.y.[50]. The Miocene/Pliocene boundary has been shown above to be about 5 m.y. old, so that the *Sphaeroidinellopsis* Acme Zone is actually correlative with the upper part of the *C. tricorniculatus* (NN12) Zone. *C. tricorniculatus* was not recorded below the top of the Messinian evaporites in the Mediterranean Sea[62]. It has been recorded, however, from the basal Messinian marls in the upper part of the type Tortonian section and the lower Pliocene Trubi Formation in Sicily, the basal Piacenzian of the Torrente Arda in northern Italy and in the type Tabianian nearby[63] as well as from levels correlated with zones N17–N19 in the Pacific core CAP 38 BP (ref. 64). The absence of *C. tricorniculatus* in the latest Messinian of the Mediterranean is probably due to unfavourable ecological conditions[50]. An overlap in the stratigraphical ranges of *C. tricorniculatus* and *C. rugosus* was observed between 625–545 cm in core CAP 38 BP (ref. 65); this interval is of early Pliocene age, equivalent to the basal part of Zone N19. In the Mediterranean the *C. rugosus* Zone was correlated consistently with the *G. margaritae evoluta* lineage Zone or with the base of the concurrent range of *G. margaritae* and *G. puncticulata* at about 4.5–4.6 m.y.[61].

The extinction of *G. margaritae* at the Gilbert/Gauss magnetic boundary (~3.32 m.y.) occurs within the upper part of the *Discoaster asymmetricus* Zone (NN14) in the Mediterranean[61]. The North Atlantic data (Leg 12) suggest that the *D. asymmetricus*/*Reticulofenestra pseudoumbilica* boundary occurs at about 3.5 m.y. (Table 3), which is slightly older than the extinction level of *G. margaritae* (~3.32 m.y.). The slight discrepancy may be due to the level of resolution we are trying to achieve here (~0.1–0.2 m.y.).

Data from the North Atlantic (Leg 12) and the Mediterranean Sea (Leg 13) agree well with the suggested ages for the various calcareous nannoplankton datum levels (Table 4) with the exception of the *R. pseudoumbilica* (extinction) Datum at 2.4 m.y. This level occurs near the bottom of Zone N21[66] and below Datum V (*Sphaeroidinellopsis* extinction) at the top of the Mammoth Event, about 2.95 m.y.[14], in DSDP core 3/12C/4/2 near Cape Verde[67], and corresponds to the initiation of continental glaciation in the Northern Hemisphere[68]. Thus, I consider an extinction date of 2.4 m.y. as somewhat too young and, indeed, it was noted[50] that near the top of its range this species is rare and represented mostly by relatively small specimens. It may be either that the (1) extinction level of *R. pseudoumbilica* determined by Gartner[50] is too high and that the upper part of the range of this species is due to reworking or (2) that the extinction level at 2.4 m.y. is real and that previous determinations on the *R. pseudoumbilica*/*D.*

surculus boundary are incorrect due to failure to recognize the latest part of the range of *R. pseudoumbilica*. The weight of the evidence cited above suggests that the correct alternative is (1) and so I assign an age of ~3 m.y. to the *R. pseudoumbilica*/*D. surculus* boundary here.

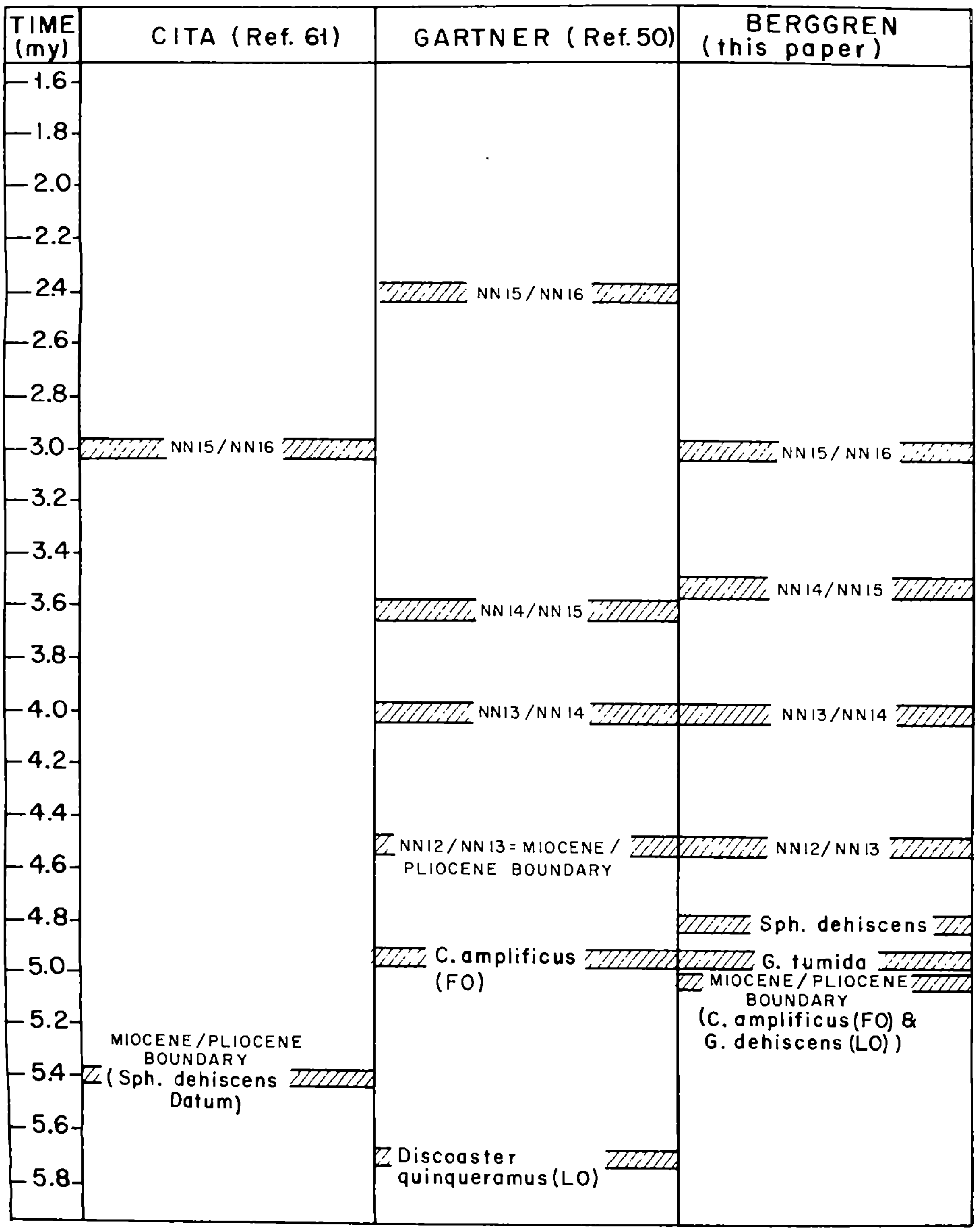
The ages of the *D. surculus*/*D. pentaradiatus* and *D. pentaradiatus*/*D. brouweri* boundaries are estimated at 2.3 and 2.0 m.y. respectively, based on extrapolated sedimentation rates (Fig. 1). Slightly different ages have been estimated by Gartner[50,51] for these levels (see Table 4) based on a slightly different interpretation of the age of the Olduvai (= Gilsa) Event. It is hoped that additional data will enable us to estimate the ages of these boundaries more accurately. A comparison of the estimated age of several of the biostratigraphical levels discussed is presented in Table 5.

Pliocene Planktonic Foraminiferal Zonation

A three-fold planktonic foraminiferal zonation (N19–N21) has been developed for the tropical Pliocene[69], but its application to biostratigraphical studies has been criticized by several authors[70–73].

Several observations on mid-Pliocene correlations in the Mediterranean region[61] are relevant to our discussion and form the impetus for the Pliocene new zonation suggested below: (1) Zone 21 (*G. tosaensis*) includes the *D. brouweri*, *D. pentaradiatus* and topmost part of the *D. surculus* Zone; (2) the base of Zone N21 lies in the upper part of the *D. surculus* Zone; (3) Zone N20 lies entirely within the *D. surculus* Zone; (4) the top of Zone N19 lies near the base of the *D. surculus* Zone, according to DSDP Leg 7 data. In the Mediterranean

Table 5 Comparison of Pliocene Datum Level Age Determinations



TIME (my)	CITA (Ref. 61)	GARTNER (Ref. 50)	BERGGREN (this paper)
1.6			
1.8			
2.0			
2.2			
2.4		NN15/NN16	
2.6			
2.8			
3.0	NN15/NN16		NN15/NN16
3.2			
3.4			
3.6		NN14/NN15	NN14/NN15
3.8			
4.0		NN13/NN14	NN13/NN14
4.2			
4.4			
4.6		NN12/NN13 = MIOCENE / PLIOCENE BOUNDARY	NN12/NN13
4.8			Sph. dehiscens
5.0		C. amplificus (FO)	G. tumida MIOCENE/PLIOCENE BOUNDARY (C. amplificus (FO) & G. dehiscens (LO))
5.2			
5.4	MIOCENE/PLIOCENE BOUNDARY (Sph. dehiscens Datum)		
5.6			
5.8		Discoaster quinqueramus (LO)	

the top of the *Sphaeroidinellopsis subdehiscens* interval zone lies near the base of the *D. surculus* Zone and the top of Zone N19 can "be easily correlated with the extinction horizon of *Sphaeroidinellopsis* spp.".

The following points can also be made: (1) The difficulty in applying the concept of Zone N20 to biostratigraphical studies in deep sea cores has been expressed by several workers[74–78]. The definition of Zones N19 and N20 has been emended[79] so that a distinct interval occurs between the top of N19 and the extinction of *Sphaeroidinellopsis* spp. This latter event occurs near the top of the Mammoth Event, at about 2.95 m.y. and is virtually coincidental with the base of the *D. surculus* Zone[61]. But this is also within or near the top of Zone N20. The base of Zone N20, as redefined[79], is marked by the initial appearance of *G. pseudopima* and not the extinction of *Globoquadrina altispira* as originally suggested[80]. The evolution of *G. pseudopima* occurs before that of *G. tosaensis*.

(2) The base of Zone N21 (*G. tosaensis*) is defined on the basis of the first evolutionary appearance of the nominate form from *G. crassaformis oceanica*[81]. This occurs at about 3.0 m.y. and is virtually identical with the extinction level of *Sphaeroidinellopsis* spp.[82].

(3) The appearance of *Pulleniatina obliquiloculata* in the upper part of Zone N19 (ref. 83) indicates that this level is above the extinction level of *G. nepenthes* (at 3.7 m.y.) which is within N19. Thus, the position of Zone N20 would be somewhere in the interval of 3.7–3.0 m.y. approximately. Actually, experience with deep-sea cores has shown that *Pulleniatina obliquiloculata* appears at or slightly above the *Sphaeroidinellopsis* extinction Datum (about 3.0 m.y.)[84]. Thus, N20 would appear to represent a short interval of time near the *Sphaeroidinellopsis* extinction level and has been estimated to span the interval of 3.3–3.0 m.y.[84].

The point is that Zone N20, if it does exist, is below Zone N21, older than 3.0 m.y. and below (=older than) the *D. surculus* Zone, the base of which can be dated at about 3.0 m.y. also. Thus, it is probably equivalent to the upper part of the *R. pseudoumbilica* Zone (NN15). Zone 20 is shown in Fig. 1 as possibly equivalent to the upper part of Zone N19 as it is questionable whether there exists a real basis for the consistent recognition of Zone N20.

Several alternative schemes of zonation for the tropical Pliocene sequences could be suggested which would greatly improve the resolution of biostratigraphical correlation while obviating the problems of correlation which now exist because of the dubious nature of N20 and the difficulty in distinguishing Zone N18 because of its extremely short duration (~0.1–0.2 m.y.). The following intervals are suggested as useful in a zonation of the Pliocene: Zone 1—interval from the evolutionary appearance of *G. tumida* from *G. plesiotumida* (4.9 m.y.) to the extinction of *Globigerina nepenthes* (3.7 m.y.); Zone 2—interval from the extinction of *Globigerina nepenthes* (3.7 m.y.) to the extinction of *G. margaritae* (3.4 m.y.); Zone 3—interval from the extinction of *G. margaritae* (3.4 m.y.) to the extinction of *Sphaeroidinellopsis* spp. (3.0 m.y.); Zone 4—interval from the extinction of *Sphaeroidinellopsis* spp. (3.0 m.y.) to the extinction of *Globoquadrina altispira* and *G. multicamerata* (2.8 m.y.); Zone 5—interval from the extinction of *Globoquadrina altispira* and *G. multicamerata* (2.8 m.y.) to the extinction of *G. miocenica* (2.2 m.y.); Zone 6—interval from the extinction of *G. miocenica* (2.2 m.y.) to the extinction of *Globigerinoides obliqua extrema* (1.8 m.y.).

The six zones can be designated as follows: Zone Pl 1: *Globigerina nepenthes—Globorotalia tumida* Partial Range Zone. Zone Pl 2: *Globorotalia margaritae—Sphaeroidinellopsis subdehiscens* Partial Range Zone. Zone Pl 3: *Globoquadrina altispira—Sphaeroidinellopsis subdehiscens* Partial Range Zone. Zone Pl 4: *Globoquadrina altispira—Globorotalia exilis* Partial Range Zone. Zone Pl 6: *Globigerinoides obliqua* Partial Range Zone. For convenience the prefix Pl is placed before each zone to indicate that it is of Pliocene age, thus Pl 1, Pl 2, and so on.

The base of the lowest Pliocene zone, Pl 1 (*Globigerin nepenthes—Globorotalia tumida* Partial Range Zone) is equivalent to the base of Zone N18 as the base, in both instance is drawn on the basis of the first evolutionary appearance c *G. tumida* from *G. plesiotumida*.

The first evolutionary appearance of *G. tumida* is virtuall synchronous with the extinction of *Globoquadrina dehiscen* (5.0 m.y.) and these two events may be useful criteria i determining the position of the Miocene/Pliocene boundar The zonation proposed here provides continuity with the lat Miocene part of one of the standard zonation systems now i wide use[85].

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LETTERS TO NATURE

PHYSICAL SCIENCES

Anomalous Absorption in the Atmosphere for 2.7 mm Radiation

MEASURED values[1–4] of atmospheric attenuation of electromagnetic radiation in the "windows" at 2 and 3 mm are greater by factors of between two and three than is calculated from monomeric water vapour and oxygen line strengths. This is true for several theoretical formulations of collision broadening. To investigate these anomalies, we have made measurements at 110 and 113 GHz (2.73 and 2.65 mm) of the variation of zenith sky noise temperatures with atmospheric conditions, using Dicke radiometers.

Simultaneous observations at two frequencies provide spectral shape information, whereas a single frequency gives only attenuation magnitude, the variation of which is more difficult to interpret. Although more spectral information could be obtained by scanning over a range of frequencies, millimetre wave techniques are such that the time taken would be too long for atmospheric conditions to remain stable, so the two-frequency method is adopted as an effective compromise. The frequencies were chosen to correspond to the observations of Straiton and Tolbert[1,2], which showed the presence of a spectral feature at 110 GHz. This was shown to be associated with the presence of water vapour, but could not be assigned to it spectroscopically.

From the measurements of sky noise temperatures, values of total atmospheric attenuation were deduced using meteorological data from radiosonde observations taken at Crawley, Sussex. The values of total attenuation were related to the ground level water vapour density obtained from measurements at RSRS since previous experience had shown that the ground level water vapour density was well correlated with the total amount of precipitable water in the path.

A plot of the difference in attenuation at the two frequencies is shown in Fig. 1, as a function of surface water vapour density. A weighted least squares fit to the data yields

$$\alpha(113)-\alpha(110)=0.53+0.002\rho \qquad (1)$$

where the attenuation, α, is in dB, and ρ is the water vapour density in g m^{-3}. The difference is a measure of the slope of the observed spectrum, and it is compared in Fig. 1 with a calculated value represented by

$$\alpha(113)-\alpha(110)=0.28+0.005\rho \qquad (2)$$

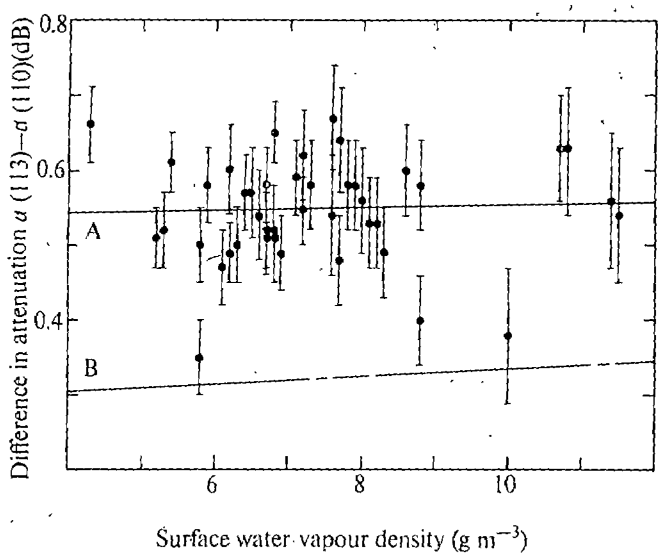

Fig. 1 Difference in attenuation at 110 GHz and 113 GHz as a function of surface water vapour density in g m^{-3}. Curve A is the least squares fit to the observations and curve B (dotted) is the theoretical prediction.

This was obtained using the Van Vleck-Weisskopf line shape for oxygen attenuation, with line-width parameters of 1.59 MHz torr^{-1} for the 60 GHz lines and 2.14 MHz torr^{-1} for the 119 GHz line. The former value was obtained by Zhevakin and Naumov[5], who showed that the Van Vleck-Weisskopf line shape provides better agreement with experiment than the Zhevakin–Naumov line shape. The value used for the 119 GHz line came from other experiments in the present series, details of which will be published elsewhere. For water vapour attenuation, the Zhevakin–Naumov line shape was used, with the half-width parameters calculated by Benedict and Kaplan[6], for quantum numbers up to 12 and for the frequency range up to 6,000 GHz.

The observed slope is approximately twice that predicted, which supports the conclusion of Straiton and Tolbert that there is anomalous behaviour at these frequencies, but the dependence of the slope on water vapour density does not agree with the earlier results. These indicated a coefficient of −0.06 in equation (1), compared with +0.002 in the present results. Some of the data in Fig. 1 apply to measurements made with thin clouds present in the field of the radiometers. The effect of these on the difference in attenuation at the two frequencies is estimated to be not more than 0.03 dB, because liquid water absorption is only 3% higher at 113 GHz than at 110 GHz. But the presence of clouds indicates that some saturated water vapour was in the path, and this could contribute to anomalous absorption.

No reasonable modification of the accepted line-width parameters of water vapour or oxygen allows the present observations to be fitted with existing theories of collision broadening. For example, use of the Zhevakin–Naumov line shape for oxygen[5] in equation (2), rather than the Van Vleck-Weisskopf line shape, only changes the first term from 0.28 to 0.31 dB. The explanation of anomalous absorption is more likely to be found in the presence of molecular complexes associated with water vapour, for which evidence has been presented from measurements at higher frequencies[7].

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Recent Freezing Level Changes and Climatic Deterioration in the Canadian Arctic Archipelago

It is well established that climatic fluctuations which are glob or hemispheric in extent are most pronounced in high northe latitudes, particularly around the North Atlantic[1-4]. Rece evidence also suggests that certain sectors of the Arctic a extremely sensitive to small climatic shifts and as a result m undergo visible changes in the landscape[5-7]. Here I report on analysis of upper air data for the Canadian Arctic archipela which indicates that marked changes in freezing level heigh have occurred during the past two decades as a result of chang in atmospheric circulation across the area.

Over most of the Canadian Arctic, surface temperatu rarely rise above 0° C from September to May. The freezi level (the 0° C isothermal surface) reaches a maximum elev tion in July. A decrease in the elevation of the freezing le during summer months can therefore greatly decrease the ar of snow and ice affected by melting.

Bradley and Miller[5] suggested that a significant clima deterioration began on Baffin Island about 1960. The freezi level data indicate that the critical change probably occurr around 1962-1964. Table 1 shows average July freezing le elevations for two nine-year periods, 1955-1963 and 1964-197 based on an analysis of daily records (no data available pri to 1955). July freezing levels have been much lower at stations in the area during the past nine years. A similar patte

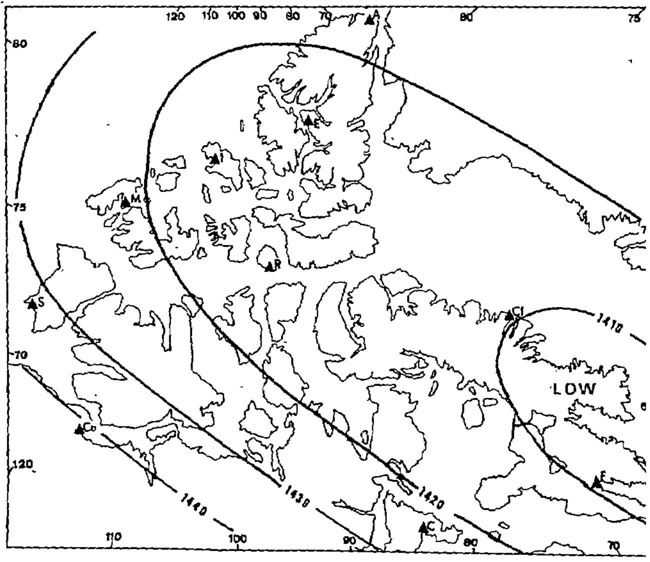

Fig. 1 Average height of the July 850 mbar surface, 1955–63, in geopotential metres (1 gpm ≃ 0.98 m). Letters refer to upper air stations mentioned in text. A=Alert; Cl=Clyde; C=Coral Harbour; Co=Coppermine; E=Eureka; F=Frobisher Bay; I=Isachsen; M=Mould Bay; R=Resolute; S=Sachs Harbour.

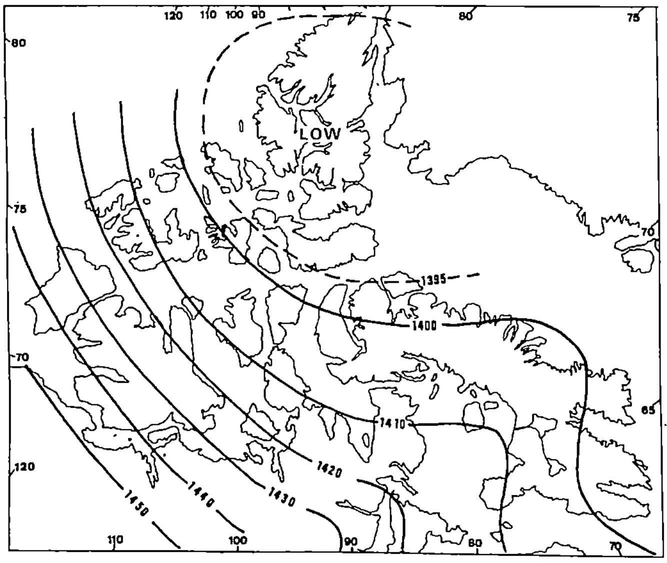

Fig. 2 Average height of the July 850 mbar surface, 1964–72, in geopotential metres.

Table 1 Average July Freezing Level Heights, 0000 GMT.* Heights in Geopotential Metres

Station	Latitude	Longitude	(*a*) Freezing level 1955–63	(*b*) Freezing level 1964–72	Change (*b*–*a*)	Student's *t*	Statistical significance
Alert	82° 30′	62° 20′	1,230	900	−330	4.9	0.1%
Clyde	70° 27′	68° 33′	1,620	1,280†	−340	4.5	0.1%
Coppermine	67° 49′	115° 05′	2,280	2,090‡	−190	2.9	0.5%
Coral Harbour	64° 12′	83° 22′	2,100	1,860	−240	3.7	0.1%
Eureka	80° 00′	85° 56′	1,500	1,150	−350	7.2	0.1%
Frobisher Bay	63° 45′	68° 33′	2,010	1,710	-300	4.9	0.1%
Inuvik	68° 18′	133° 29′	2,500§	2,370	−130	2.2	2.5%
Isachsen	78° 47′	103° 32′	1,220	710	−510	7.3	0.1%
Mould Bay	76° 14′	119° 20′	1,310	1,150	−160	2.1	2.5%
Resolute	74° 43′	94° 59′	1,490	1,150	−340	4.7	0.1%
Sachs Harbour	71° 59′	125° 17′	1,790‖	1,640	−150	1.8	5.0%

* July 1955 soundings taken at 0300 GMT.
† 1964–70 inclusive.
‡ Data 1970–72 inclusive from Cambridge Bay (69° 06′ N, 105° 07′ W).
§ Data 1955–60 inclusive from Aklavik (68° 14′ N, 135° 00′ W).
‖ 1956–63 inclusive.

is apparent for the summer months as a whole. A maximum freezing level depression of 510 geopotential metres occurred at Isachsen in the northwestern archipelago with other large decreases in a broad belt north to Eureka and Alert and southeast to Resolute, Clyde and Frobisher Bay.

Mean pressure contour maps for the same periods (Figs. 1 and 2) indicate that during the former period the area was dominated by a trough of low pressure over Baffin Bay and southern Baffin Island. In the subsequent period the principal low pressure centre occurs to the northwest with the southeastern Arctic dominated by a ridge of high pressure. Pressure gradients over the archipelago are also much stronger in the latter period indicating increased movement of air across the region, particularly from the north. A similar pattern of circulation change is observed at the 700 mbar level. The low pressure centre of 1964-72 is located precisely where the most significant falls in freezing level have occurred (Table 1). Increased advection of cool air across Baffin Island during the latter period accounts for the relatively large falls at Clyde and Frobisher Bay. Relatively small changes over the southwestern archipelago reflect little change in the circulation over that area.

The large changes in summer freezing levels across the area thus seem to be the result of changes in synoptic conditions. Namias[8] suggests that a "new climatic regime" began late in 1961 as a result of anomalously warm ocean temperatures in the central northern Pacific Ocean which had the effect of amplifying the standing long wave pressure pattern in the Northern Hemisphere. The observed displacement of the main centre of the eastern Canadian trough north and west is consistent with Namias's suggestions. The abrupt changes in freezing levels around 1962-64 reflect a lag in response to the initiation of this new circulation regime.

The large changes in freezing level height since 1963 have produced significant glaciological responses in the Canadian Arctic. Observations made in 1967 in the United States Range, central Ellesmere Island, led Hattersley-Smith[9] to conclude that the summers of 1963-66 "were the coldest sequence of summers since before 1925 . . . the equilibrium line (ELA) on the glaciers had been lowered to approximately 900 metres from a mean of about 1,200 metres in the years 1957-63". This fall in ELA by 300 m is similar to the drop in freezing level at Alert and Eureka (Table 1). Similarly, the ELA on the White Glacier, central Axel Heiberg Island, has been more than 400 m lower since 1963-64 than in the four years 1959-60 to 1962-63 (ref. 10). Again a similar fall in freezing level height over this area has been noted. Finally, a recent study of sea-ice extent in Baffin Bay and Davis Strait shows that a very marked change towards more severe ice conditions occurred in 1963 (ref. 11). Between 1952 and 1960 there were only four years when significant areas of ice were observed in September whereas "in the 1960s there was only one year in which ice was not present in September, and five years in which it survived until October". Such a deterioration clearly reflects the lower temperatures and increase in northerly flow over the area during the past decade.

In conclusion, the Canadian Arctic has recently experienced a significant climatic deterioration. Any further fall in freezing level heights or the persistence of recent conditions will undoubtedly result in increased glacierization throughout much of the eastern and northern upland area. This indicates the importance of carefully monitoring climatic conditions in the Canadian Arctic archipelago over the next few years.

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Compensation Law in Thermodynamics and Thermal Death

THE isokinetic relationship or, more generally, the compensation law, a linear relation between enthalpy and entropy, has been debated for many years. Evidence for it occurs in phenomena such as chemical reactions, solubility, evaporation of metals, catalytic processes, thermal denaturation of macromolecules, thermal killing of unicellular organisms, and semiconduction processes. Such a widespread occurrence advises avoidance of *ad hoc* explanations, and suggests a common denominator on a thermodynamic or statistical mechanical basis.

Objections raised to the validity of a compensation law fall into four categories, namely, (1) that there are inadequate data for a sufficient range of temperatures and number of Arrhenius lines; (2) that logarithmic compression accidentally produces an apparent compensation law; (3) that biased sets of data are chosen; and (4) that such a law cannot, at present, be derived from first principles of statistical mechanics, or thermodynamics. The objections recently raised by Banks *et al.*[1] fall into categories (2) and (3). They are correct to point out the dangers in simply plotting the measured values of $\Delta H^\ddagger$ from Arrhenius plots against the calculated values of $\Delta S^\ddagger$ from the transition rate theory equation, because both are proportional to the logarithms of the rate constants. Also, because of experimental bias in favour of a comfortable range of reaction rates, and the biological bias that death rates of unicellular organisms should be measured in times less than cell division times, one may accidentally pick out a subset of protein denaturation rates and death rates which provide a subset of $\Delta H^\ddagger$ and $\Delta S^\ddagger$ values falling in the same narrow strip of the $\Delta H^\ddagger$–$\Delta S^\ddagger$ plane.

An entirely different argument inverts the argument of Banks *et al.* This refers to Arrhenius plots the slopes of which yield very high activation enthalpies. For reaction rates, activation enthalpies greater than 50 kcalorie mol^{-1} are fairly common. Conventional pre-exponential factors cannot possibly be large enough to offset these extremely small exponential values in order to yield the measured rates. Thus, in order for such reaction rate constants to be measurable, there must be another, positive, exponential term added to the Arrhenius equation. If the activation enthalpy can be varied over a wide range of values, this positive exponential factor must also vary with it and thus this factor must also be a function of the activation enthalpy and must be independent of temperature. Such a term is $\exp(\Delta S^\ddagger/R)$ where $\Delta S^\ddagger$ is proportional in some way to $\Delta H^\ddagger$. This is a compensation effect. The simplest, though not necessarily correct, proportionality is a linear relation $\Delta S^\ddagger = a\Delta H^\ddagger + b$. This is one form of a compensation law. We conclude from this that the only physically detectable processes of high activation enthalpies are those where a positive exponential factor operates, and if the activation enthalpies vary over a sufficiently wide range, the compensation law must be valid. If the enthalpies only vary over a small range one must exercise extreme caution and apply a rigid criterion of validity before accepting or rejecting the hypothesis of the operation of a compensation law[2]. For a very wide range of activation enthalpies, empirically determined from good Arrhenius plots, one must accept the operation of a compensation law. Because the thermal denaturation of proteins and the thermal inactivation or death of unicellular organisms contain a majority of cases of very high activation enthalpies, but also include lower ones, these must be assumed to be compensation law processes[3].

There are many ways to evaluate a and b in the linear $\Delta S^\ddagger$, $\Delta H^\ddagger$ relation. The simplest is to plot $\Delta S^\ddagger$ against $\Delta H^\ddagger$ for the appropriate series. Logarithmic compression now minimizes the fluctuations in the rate constant data. Thus $T_c(=1/a)$ and b can be evaluated fairly accurately and used for correlational purposes[3]. The range of variation of $\Delta H^\ddagger$ must be larger than about 50 kcalorie mol^{-1} if this method is used to establish the validity of the compensation law. The data for protein denaturation and thermal deaths cover a range of $\Delta H^\ddagger$ of about 150 kcalorie mol^{-1}.

We agree that large values of $\Delta H^\ddagger$ are likely to be associated with complex processes, because unit steps do not involve so much enthalpy. Protein denaturation is certainly a complex process. Such processes, however, can also be described by the absolute theory of rate processes. The problem of combining unit steps into a complex process, which in turn also looks like a unit process, has also been treated by one of us (G. K., unpublished). The theory of rate processes can be used, contrary to Banks *et al.*, in the gas, liquid, or solid phases. In complex cases, we may not be able to assign an origin to $\Delta H^\ddagger$ and $\Delta S^\ddagger$, but this does not mean that these quantities are meaningless. Although there is a difference in practice between "unit steps" and complex processes, we see no difference in principle, because "unit steps" (that is, breaking of one chemical bond) are themselves due to multiple steps of additions of vibrational energy.

Even if the compensation law for complex processes were not quantitatively accurate, there must be a general trend that larger values of $\Delta H^\ddagger$ are associated with larger values of $\Delta S^\ddagger$. As $\Delta H^\ddagger$ increases for a reaction series, this increasing enthalpy must be shared out among an increasing number of units. The larger the number of units, the larger will be the density of states associated with the activation. This leads to the rough proportionality between $\Delta H^\ddagger$ and $\Delta S^\ddagger$. This line of reasoning leads to a statistical mechanical interpretation of the compensation law and of the compensation temperature (G. K. and Goklany, unpublished).

Banks *et al.* state that because bacterial cells are either alive or dead, death is necessarily a first order kinetic process, as in radioactive decay. That is not true. Plots of the logarithm of the mortality rate as a function of time sometimes exhibit an initial shoulder before a straight line portion. This may be attributed to multiple hit requirements, as in radiation effects, or repair mechanism of some damage. We have excluded these cases. Multicellular organisms including *Drosophila*, mammals and man may also be either alive or dead. In these cases, it is known that the kinetic rate constants are time dependent, following a Gompertzian function. It is not yet known at which stage in the transition from unicellular to higher multicellular organisms, the kinetics change from the exponential to the Gompertzian.

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Thermodynamic Compensation Rule

Banks, Damjanovic and Vernon[1] have proposed that the thermodynamic compensation rule which is claimed to apply to the denaturation of proteins[2,3] is the result of an experimental artefact and thus of no physical significance. In particular, by taking logarithms of the unimolecular rate equation, they obtain

$$\log C - \log kT/h = \Delta S^{\ddagger}/2.303R - \Delta H^{\ddagger}/2.303T \qquad (1)$$

where C is the rate constant of the reaction. Provided that $\log C$ does not vary very much, for a small range of temperatures the left side of equation (1) is approximately constant so that $\Delta S^{\ddagger}$ and $\Delta H^{\ddagger}$ are linearly related. But the real point of physical significance is not so much that $\Delta S^{\ddagger}$ and $\Delta H^{\ddagger}$ are linearly related, but that $\log C$ should vary so very little with $\Delta H^{\ddagger}$. In the particular case they consider, this is the result of the experimental difficulty of measuring more than a small range of values of C and T so that only those materials which apparently obey the compensation law are susceptible to investigation.

The compensation law is also observed, however, in the electrical conductivity of organic semiconductors[4] and amorphous semiconductors[5]. In those cases the measurable range of conductivity, σ, extends over at least twenty orders of magnitude so that any constancy of σ at a particular temperature would be of real physical significance. In the cases cited this critical temperature is much higher than the experimental range of temperatures investigated, so that at the experimental temperature, the left side of equation (1) is no longer constant. In the case of amorphous semiconductors the critical temperature is greater than 1,000° C. No doubt with a suitably wide and uncorrelated variation in $\Delta H^{\ddagger}$ and σ it would be possible to group together materials such that they apparently obey a compensation law. It is, however, physically significant that the groupings so obtained correspond to the groupings that would have been made on chemical or structural grounds.

Although a number of explanations of the compensation law for conductivity have been proposed[6-9], there remains the possibility that this is also an experimental artefact[10]. But it is clear that the particular explanation proposed by Banks *et al.* cannot be appropriate in this case.

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Compensation Effect and Experimental Error

Banks, Damjanovic and Vernon[1] suggest that the observed compensation effect in the rates of some biological processes might be wholly artefactual. Their argument is one which would also apply to systems of purely chemical interest and raises the question of whether, in the inevitable presence of experimental errors, a true compensation effect is ever observable.

I use the term compensation effect to describe the systematic variation of the pre-exponential factor, A, with the activation energy, E, in the Arrhenius equation

$$k = A\exp(-E/RT) \qquad (1)$$

often observed for a series of similar reactions. Here k is the specific rate constant, T the temperature and R the gas constant. When a variation is observed, it is usually[2] found to have the form

$$\log A = \frac{E}{2.303RT_s} + \log k_0 \qquad (2)$$

the parameter T_s has units of temperature and corresponds to the temperature at which all the reactions of the series proceed at the same rate, k_0.

Cremer[3] discussed the possibility that compensation effects may arise out of experimental errors. For simplicity I consider separately the effect of uncertainties in E and k on $\log A$. Rearranging equation (1) and taking logarithms

$$\log A = \log k + \frac{E}{2.303RT} \qquad (3)$$

For an uncertainty δE in E there will be an associated uncertainty $\delta \log A$ in $\log A$:

$$\log A \pm \delta \log A = \log k + \frac{E \pm \delta E}{2.303RT} \qquad (4)$$

Comparing this with equation (2) it is seen that the range of

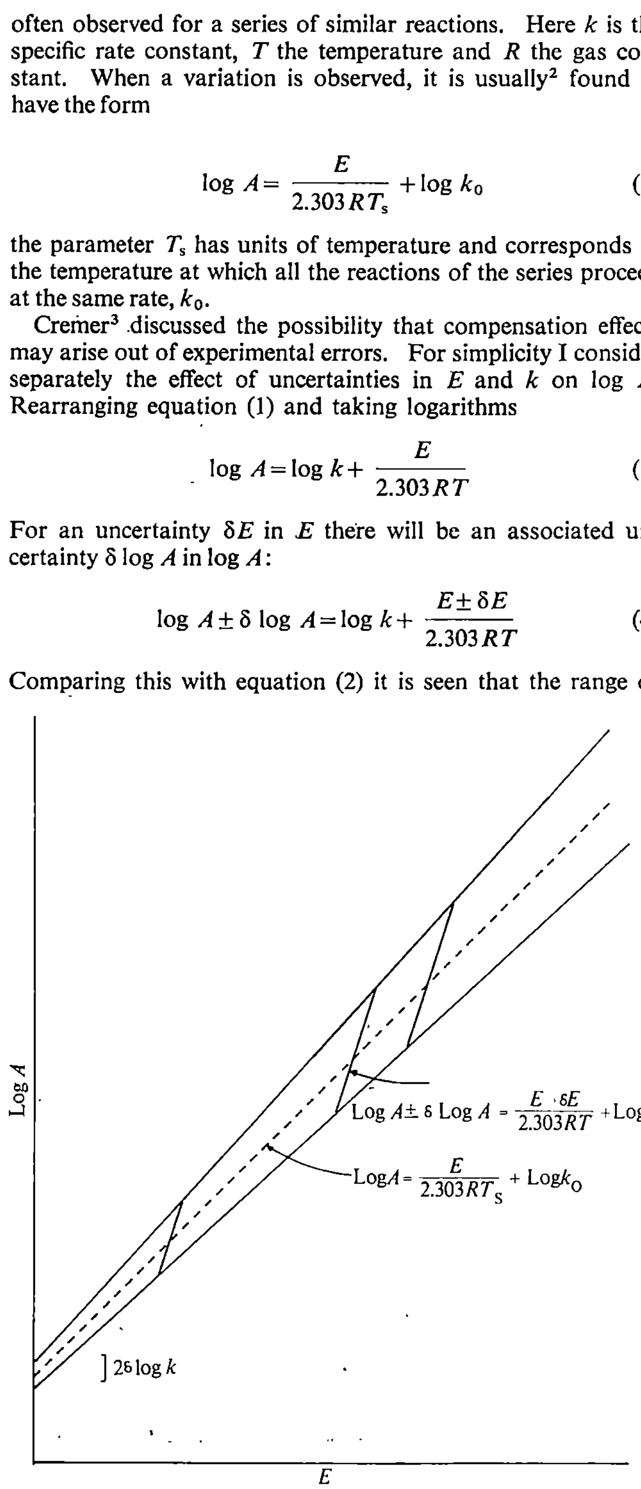
Log A
$\text{Log}\, A \pm \delta\, \text{Log}\, A = \frac{E \pm \delta E}{2.303RT} + \text{Log}k$
$\text{Log}A = \frac{E}{2.303RT_s} + \text{Log}k_0$
$2\delta \log k$
E

Fig. 1 Range of expected compensation effect in the presence of experimental error.

values of $E \pm \delta E$ and $\log A \pm \delta \log A$ are related by a compensation effect with $T_s = T$. But the uncertainty in $\log k$ of $\delta \log k$ has an associated uncertainty in $\log A$ of identical magnitude independent of E. In a plot of experimentally observed values of $\log A$ and E for a particular reaction, therefore, the experimental temperature determines the slope and the uncertainty in $\log k$ determines the scatter. The fit to the compensation effect plot is independent of the uncertainty in E, as was found by Banks, Damjanovic and Vernon[1], but the spread of activation energies covered by it is only $2\delta E$. Because it is k which is measured experimentally and $\delta \log k$ which is responsible for the scatter, compensation effect plots of this kind would usually appear to be obeyed very well.

I now consider the experimentally observed compensation effect for a series of reactions in which E and A are intrinsically related by equation (2). Taking δE and $\delta \log k$ as the uncertainties in E and $\log k$ as before,

$$\log A \pm \delta \log A = \frac{E}{RT_s} \pm \frac{\delta E}{RT} + \log k_0 \pm \delta \log k \qquad (5)$$

This represents an area in the compensation effect plot bounded by the envelope of all of the compensation effects due to errors in E and $\log k$ for all the individual reactions. The shape depends on the relative magnitudes of T and T_s in equation (5) and on the relation between δE and E. In Fig. 1 I show the case where δE is proportional to E. Clearly as T approaches T_s the fit to equation (2) improves, and when $T \neq T$ the magnitude of δE becomes important.

I conclude that if the range of an experimentally obtained compensation effect exceeds twice the uncertainty in the activation energy then there probably exists an intrinsic relation between A and E. In the absence of large systematic errors the parameter T_s is obtained from the slope of the compensation effect plot. Only the quality of the plot is dependent on the magnitudes of the errors and it is the uncertainty in E which assumes greatest importance at temperatures much different from T_s.

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REPLY to Boon and to Harris: We are well aware that the so-called compensation law behaviour has been observed in the case of the electrical behaviour of semiconductors, and as we indicated, may not be wholly artefactual in some cases in physical organic chemistry. We were merely concerned to show that the experimental data on the denaturation of proteins and the thermal death of viruses and microorganisms cannot be used to adduce compensation law behaviour in these cases. The linearity of the plot of $\Delta H^\ddagger$ against $\Delta S^\ddagger$ arises wholly from the near constancy of the left hand side of the equation used in calculating $\Delta S^\ddagger$ from values of $\Delta H^\ddagger$ which vary between 10 and 200 kcalorie mol^{-1}. It is unlikely that the uncertainty in the experimentally determined activation energies for protein denaturation is greater than half the range over which compensation law behaviour is observed so the analysis put forward by Harris does not seem to cover all cases where compensation law behaviour is artefactual.

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Compensation Effect in Heterogeneous Catalytic Reactions including Hydrocarbon Formation on Clays

A SERIES of comparable reactions is said to exhibit compensation behaviour when the Arrhenius plots show systematic variations of the activation energy (E) and the pre-exponential factor (A) according to the expression

$$\log A = bE + c \qquad (1)$$

Many examples of such behaviour have been reported for diverse reaction systems, but hitherto no explanation of the effect has been generally accepted. Indeed, it has been suggested[1–3] that obedience of data from a sequence of reactions to equation (1) may arise through the method of analysis of the kinetic measurements or it may be an artefact. But before compensation behaviour is dismissed as a phenomenon of little theoretical significance, we suggest that in at least one field, heterogeneous catalysis, obedience to equation (1) may be considered to result from a useful mechanistic model. Many examples of compensation behaviour among reactions catalysed by solids have been reported and Bond[4] has discussed the theoretical implications. The present preliminary report describes a further sequence of surface rate processes which exhibit the compensation effect and proposes a model to explain these observations.

Alcohols and hydrocarbons react[5] on montmorillonite surfaces to yield a characteristic mixture of hydrocarbon products in which *iso* and *anteiso* alkanes predominate. The mechanism of these reactions, proceeding under conditions of possible primary product and water vapour readsorption[5], includes polymerization, cracking, isomerization and hydrogen transfer steps. We have now completed a study of the reactions of *n*-dodecanol and of stearic acid on the clay minerals illite, kaolinite and montmorillonite. These reactions yielded product hydrocarbon mixtures similar to those referred to above[5] and will be described elsewhere. The present report is restricted to the consideration of two particular observations for the six reactant mixtures studied: (1) The A and E values exhibit compensation behaviour (Fig. 1); (2) kinetic data, measured for comparable reaction rates, were obtained at similar temperatures (within the approximate range 420–550 K).

From comparison of the product mixtures, we conclude that the reactions to which Fig. 1 refers proceeded by comparable mechanisms involving[5] the rearrangement of similar mixtures of hydrocarbon fragments bonded to the clay surfaces. In spite of crystallographic differences between the three mineral phases, the catalytic properties of the active surfaces, composed of somewhat different arrays of oxide and hydroxide ions[6], were closely similar. Significantly, these similarities include the appearance of detectable catalytic activity at temperatures slightly below the range of onset of bulk dehydroxylation. Accordingly, we associate the participation of clays in reactions of organic compounds with the reactivity and mobility of active constituents of the mineral surface. Such surface species undoubtedly include protons and hydroxyl groups which are involved in the dehydration reaction; water loss, in common with many reactions of solids, probably proceeds preferentially at crystal surfaces. The same groups may also be expected to participate in the dissociative adsorption and subsequent surface catalytic rearrangements during which *n* dodecanol, or stearic acid, was converted to hydrocarbons. We selected 2-methyl hexane as the representative product for which kinetic data are reported here (Fig. 1).

To discuss a possible mechanistic explanation of compensation behaviour, we write the expression for the rate of product (P) formation from a heterogeneous reaction in the form[4]

$$\mathrm{d}P/\mathrm{d}t = \nu\sigma S_{\mathrm{H}} S_{\mathrm{C}} \exp(-E'/RT) \qquad (2)$$

where ν is a surface vibration frequency, σ includes entropy and orientation factors and S_{H} and S_{C} are the effective surface concentrations of the hydrogen and the hydrocarbon fragment which react on the catalyst in the step which controls the rate of product formation. We wish to consider the significance of the magnitude of $S_{\mathrm{H}}S_{\mathrm{C}}$ on the observed kinetic behaviour. A temperature dependence of this term, across the range for which rate constants are measured, must influence the value of the apparent activation energy. For example, the value of E determined for a reaction in which the change in the magnitude of $S_{\mathrm{H}}S_{\mathrm{C}}$ is the same as that resulting from the exponential temperature dependence is O or $2E'$, depending on the direction of the change. It is reasonable to suppose that such changes in the availability of surface reactant species with temperature do occur, due to surface equilibria, including the heat of reactant adsorption. It may be expected therefore that there would be different rates of variation of $S_{\mathrm{H}}S_{\mathrm{C}}$ with temperature in a sequence of different reactions proceeding by similar mechanisms within a comparable temperature interval and having equal values of ν, σ and E'. Quantitative consideration of the effect of such variations in the availability of those species which react in the rate limiting step indicates that a compensation effect will occur for the measured values of A and E. Further, the range of values of A derived from such considerations extends beyond the limits expected for a simple surface reaction, but which have been found in experimental measurements (for example, $10^{22} < A < 10^{34}$ mol m^{-2} s^{-1}, Fig. 1). In the following discussion we consider application of this model to the compensation effect in heterogeneous catalysis.

The estimation of the concentrations of particular surface species included in equation (2) (S_{H} and S_{C}) may be difficult, if not completely inaccessible to experimental measurement, because the identification of the participants in the rate limiting step of a surface process is not always possible. Active intermediates may occupy only a small fraction of the catalyst surface[7]. Heterogeneous reactions often proceed at a temperature range within which there may be extensive dissociative adsorption of the reactant molecules. Surface coverage may be incomplete due to desorption of the reactants and/or products. For example, during hydrocarbon cracking reactions on nickel (a series of reactions which exhibits[8] compensation behaviour) there may be dissociation of chemisorbed methane (and other hydrocarbons) and hydrogen desorption (ref. 8 and articles cited therein). Thus the complexity of the surface dissociation processes and interdependent equilibria in many systems of interest makes it difficult, if not impossible, to estimate meaningfully S_{H} and S_{C}. The majority of chemisorption measurements have, for obvious reasons, been concerned with processes occurring below the temperature range of rapid catalytic reactions. There is no reason for an *a priori* assumption that the product $S_{\mathrm{H}}S_{\mathrm{C}}$ should invariably remain constant within the conditions used for an activation energy measurement. It seems more probable intuitively that there would be changes in surface equilibria, perhaps involving exponential variations of S_{H} and of S_{C} with reciprocal temperature.

We emphasize that S_{H} and S_{C} represent effective concentrations of surface species; this must include the influence of surface mobility on the reaction frequency factor. At low temperature each reactant species is firmly anchored at a particular site, so that the probability of a collision leading to reaction is negligibly small. On raising the temperature, the probability of contact between reactants is increased by surface migration, but the meaningful estimation of a collision number is impracticable. Surface movements, representing a redistribution of chemisorption bonds, must be an important factor in the reactivity of adsorbed species. We have shown that the catalytic properties of the clays become significant in the temperature range immediately below that at which interaction between surface groups leads to mineral dehydration. Thus there is here an association between surface reactivity and compensation behaviour.

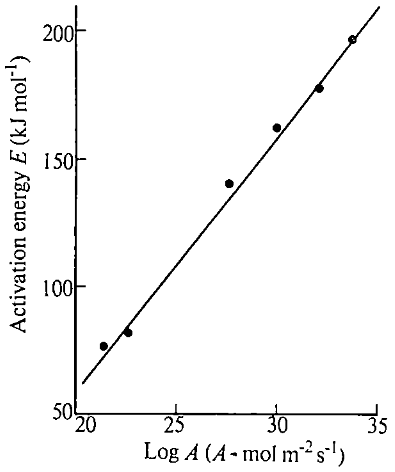

Fig. 1 Compensation plot for hydrocarbon formation reactions on clays. Values of A and E obtained from measurements of the rates of 2-methyl hexane evolution during the reactions of *n*-dodecanol and of stearic acid on illite, on kaolinite or on montmorillonite.

Compensation effects are usually described for a sequence of chemical reactions in which one or more factors, such as reactants, products, catalysts and possibly the rate limiting process, are common to each reaction within the group which exhibits the effect. Examples include hydrocarbon formation reactions on clays (Fig. 1), hydrocarbon cracking reactions on nickel[8], methane exchange reactions on alloys[9], the decomposition of formic acid on silver[10] and others[4]. The chemical changes occurring in the reactions of each group are closely comparable, but surface equilibria, and thus concentrations of the intermediates, may be influenced (through the heat of adsorption) by catalyst structure (clays, alloys[9], and surface structure of silver[10]) or the different organic reactants (cracking on nickel[8]). Pétermann[11] has ascribed compensation effects in the desorption of hydrogen from metals to temperature variations of transmission coefficients.

Compensation behaviour can therefore be provided with a mechanistic basis in which there are different variations in the temperature dependence of the effective concentrations of the surface species which interact during the rate-limiting step for product formation in a sequence of comparable reactions. Such changes in reactant availability result from differences in surface equilibria and heats of adsorption, reactant dissociation, distribution and mobility. Features of this model, involving variations of the concentrations of intermediates, may also be applicable to the theoretical consideration of homogeneous reactions which exhibit the compensation effect.

The data in Fig. 1 extend previous kinetic studies of reactions which may participate in petroleum genesis[12,13]. Two general points may be made:

First, the compositions of the mixtures of product hydrocarbons from all six reactant mixtures were closely comparable, thus indicating the conversion of organic compounds to hydrocarbons by the same mechanism (discussed previously[5]) on the different mineral surfaces. The catalytic activity is therefore associated with similar surface sites on the several alumino-silicates.

Second, the Arrhenius parameters for hydrocarbon formation from the different reaction mixtures showed considerable variations, and the system quantitatively considered in the geochemical context[13] (*n*-dodecanol and montmorillonite) showed the largest dependence of rate on temperature. Thus, if the extrapolation used[13] to estimate hydrocarbon formation in sedimentary deposits at lower temperatures is applicable, this particular mixture of reactants must be regarded as the least active of the series currently investigated. The conclusion that clay/organic compound mixtures provide a feasible route for the catalytic production of hydrocarbons in nature is further supported by the present observations. Indeed, the indications from the kinetic study are that illite and kaolinite may be more active catalysts than the montmorillonite first studied.

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BIOLOGICAL SCIENCES

Release of ATP from Rat Motor Nerve Terminals

INVESTIGATION of the adrenal medulla[1,2] and the *Torpedo* electroplax[3] suggested that ATP plays some part in the storage and release of chemical transmitters. The fluid bathing contracting amphibian muscle contains ATP, but this ATP was thought to be released from the muscle[4]. We present here evidence that ATP is released from motor nerve terminals on indirect stimulation of a mammalian nerve-muscle preparation.

To detect ATP we used our bioluminescence injection-housing system, described elsewhere[5]. The sensitive photomultiplier utilizes a bialkali photocathode and is capable of detecting as little as 9.2×10^{-11} g ATP per ml saline when exposed to crude commercial firefly lantern extract (Sigma). As Fig. 1 shows, opening the shutter of the housing system (Fig. 1, S) reveals the resting luminescence of the extract (Fig. 1, L) due to endogenous ATP. On injection of the indicated concentrations of ATP in 250 µl of solution (Fig. 1, arrow) a chloride-induced inhibition of the resting luminescence of the extract occurs followed by the classical light emission reaction[6]. The peak of light emission serves as a linear quantitative assay for ATP. The baseline for measurements of light emission was taken as the inhibited level after injection of ATP-saline (Fig. 1, arrow). Our particular assay enables ATP to be distinguished from ADP and from other nucleoside triphosphates, as each nucleotide has a different temporal pattern of light emission.

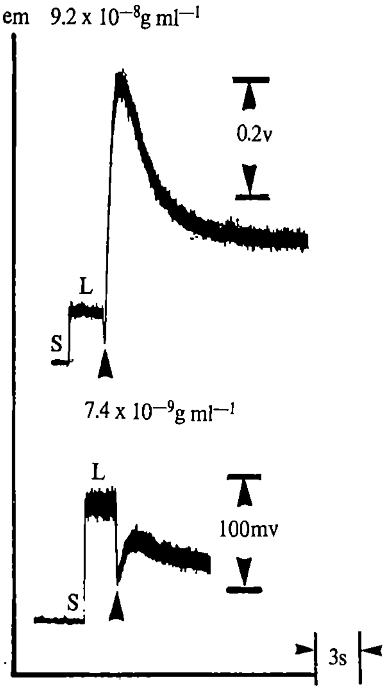

Fig. 1 Chart record of the light emission after injection of 250 µl of physiological saline solutions of ATP into 430 µl of firefly lantern extract. Additional details in text.

Our preparation was the left phrenic nerve-hemidiaphragm of white albino rats, carefully dissected to avoid cut muscle, pinned in a lucite chamber and stimulated indirectly through a polyethylene suction electrode. Preparations were bathed

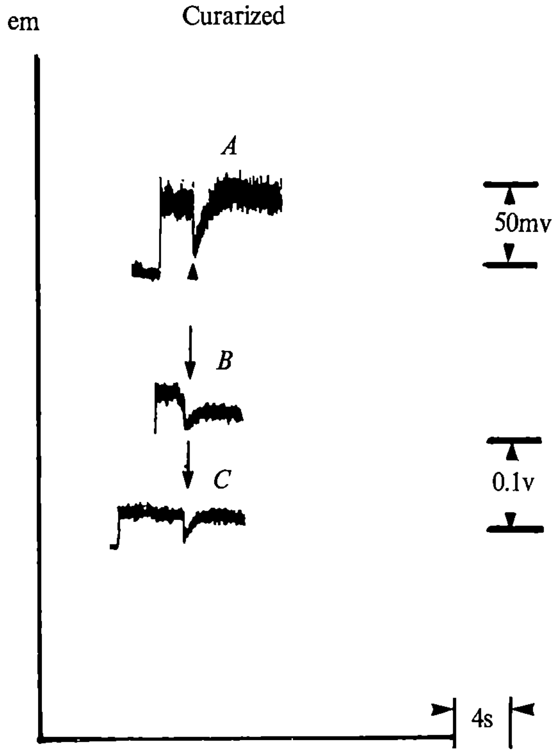

Fig. 2 Chart records of light emission after injection (arrows) of 250 µl samples of saline taken from the solution bathing a curarized preparation which was either indirectly stimulated (*A*, *C*) or rested for 20 min (*B*). In (*A*), stimulation was at 8.5 Hz for 15 min and the preparation was soaked for 5 min before the sample was taken. In (*C*), stimulation was for 20 min at 9 Hz. Note that sensitivity for *B* and *C* is half that for *A*. The light emission recorded in *A* is equivalent to that produced by approximately 6×10^{-7} g ml^{-1} ADP.

in the modified physiological saline of Liley[7] at room temperature.

Preliminary investigations on contracting muscle using a protocol similar to that of Boyd and Forrester[4] (washing for 10 min, nerve stimulation at 2 Hz for 25–30 min and soaking for 5 min) yielded essentially the same quantity of ATP in the perfusion fluid as was detected in their amphibian studies. It was found, however, that in the rat preparation the nucleotide efflux for the 35–40 min period investigated was actually a washout of extracellular ATP and therefore not related to the contractile process. Indeed, in none of seven stimulus-control periods in four separate preparations was there any stimulus-specific release of ATP detectable in the bath of the contracting diaphragm, even if the preparation was first perfused for 1–2 h before nerve stimulation at 2 Hz.

Release of ADP, associated with nerve stimulation in the absence of contraction, however, could be detected. Figure 2 shows the results from a preparation curarized to a level at which no end-plate potentials could be detected. In this instance, approximately six times as much ADP was present in the bath after stimulation (Fig. 2*A* and *C*) as in the control period (Fig. 2*B*). The perfusion fluid at the time of assay was devoid of ATP.

It seemed probable that the absence of ATP in the effluent indicated the presence of nucleotidases activated by Ca^{2+} and Mg^{2+} (refs. 8 and 9). This hypothesis was supported by the results of two experiments in which, when the normal bathing 2 mM (Ca^{2+}) was raised to 5 mM, neither ATP nor ADP was detectable in the bathing fluid of the preparation. To minimize this complication, preparations were bathed in solutions with no added Mg^{2+} and only 0.1 mM Ca^{2+}. ACh release was then effected by exposure to a solution made hyperosmotic by the addition of sucrose and by nerve stimulation. In such conditions of hypertonicity these preparations show a large increase in spontaneous ACh release[10]. As Fig. 3 hy shows, 30 min after the beginning of perfusion with the hyperosmotic solution (500 mosM), ATP efflux was increased to a constant level 3.1 times control values. A similar increase in spontaneous ACh release would be expected[10] at this time. On indirect stimulation (Fig. 3 hy, Stim 14 s^{-1}), a 29% increase in the amount of ATP in the bath was observed.

These results are strongly indicative of ATP release from nerve terminals. Two additional lines of evidence support this suggestion. First, when preparations were bathed in less hyperosmotic solutions (400 mosM), only a doubling of ATP efflux was observed. On stimulation, however, a larger increase in ATP release occurred in the 400 mosM solutions (mean of 43% in three experiments) than in the solutions of higher osmolarity (Fig. 3). This difference would be expected if the nucleotide were associated with transmitter release, for in the presence of 500 mosM solutions there is a decline in the amount of ACh released by nerve impulses after exposure for 8–10 min[10]. Second, when the experiments were repeated in the absence of Ca^{2+}, which would prevent ACh release by nerve impulses, no increase in ATP efflux occurred on nerve stimulation in the hyperosmotic solutions.

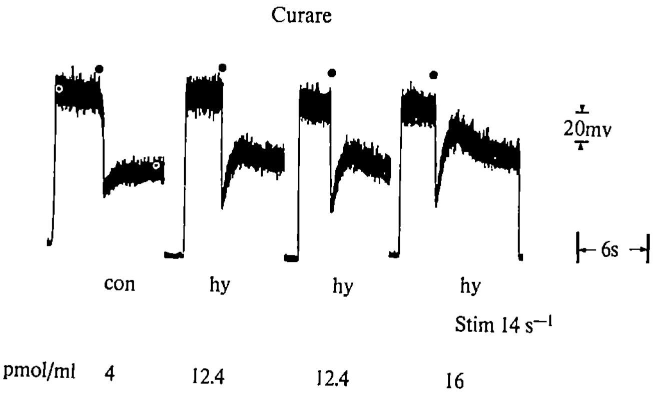

Fig. 3 Chart records of light emission evoked by samples of the fluid bathing a preparation exposed to either a hyperosmotic solution (hy) or to a hyperosmotic solution and nerve stimulation (hy, Stim) in the absence of added Mg^{2+} and 0.1 mM Ca^{2+}. The preparation was curarized. Actual concentrations of ATP in the bath are indicated beneath each record. Control (con) represents ATP efflux before the addition of hyperosmotic solutions; hy, two consecutive 10 min determinations of bathing fluid ATP concentration after the preparation was first exposed to hyperosmotic (500 mosM) solutions for 30 min; hy, Stim 14 s^{-1}, bathing fluid following nerve stimulation at 14 Hz for 10 min. Dots indicate the time of sample injection. Additional details in text.

Additional experiments suggested that the ATP was not derived from nerve above the terminal; as shown in Fig. 3 repetition of the experiments on phrenic nerves revealed no difference in ATP release in any of the experimental situations. This does not, however, eliminate the possibility that diaphragm ATPases were inhibited in hyperosmotic solutions or that some non-specific effect of hypertonicity was contributing to the increase in ATP efflux illustrated in Fig. 3 hy.

All preparations in the hyperosmotic studies were perfused for 2.5 h before the assay of effluents, and contracted in response to nerve stimulation when returned to a normal bathing solution at the end of an experiment.

We suggest that ATP released by nerve stimulation comes from nerve terminals. This ATP could play some part along with protein in the complexing of ACh in the storage vesicle and may also have a trophic function[3].

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Thermal Synthesis of Amino Acids from a Simulated Primitive Atmosphere

IT has been suggested that thermal energy was readily available for the prebiotic synthesis of organic compounds as evidenced by lava remnants from earlier volcanic activity and by the present widespread occurrence of regions of elevated temperatures on the Earth[1,2]. In previous chemical evolution thermal experiments, the predominant amino acids reported were those commonly found in present-day protein[1,3,4]. It was implied from these results that thermal energy may have played an important part in the prebiotic synthesis of protein amino acids. It would certainly be significant if thermal energy could produce only these amino acids and not the unusual amino acids of the type identified in simulated primitive atmosphere discharge experiments[5,6] and in meteorite extracts[7,8], a presumed extraterrestrial abiotic synthesis. Also, previous studies have relied on the identification of amino acids by ion exchange chromatography[1,4] (amino acid analyser) and electrophoresis[3], which is not sufficient for the unequivocal identification of an amino acid, particularly when materials other than the common amino acids may be present[5,9]. For these reasons we have applied the recently developed techniques of gas chromatography (GC) and gas chromatography combined with mass spectrometry (GC–MS) for identification of the compounds produced by thermal synthesis.

The synthesis was carried out by passing methane (120 ml min^{-1}), which was bubbled through water at 80° C, and ammonia (60 ml min^{-1}) through a 'Vycor' tube containing quartz sand. Reactions were performed with the tube at various temperatures between 900° C and 1,060° C while controls were held at ambient temperature. The gases from a run of 2–3 h were collected in 3 N aqueous ammonia (maintained in an ice bath) and then subjected to a temperature of 75° C for 35 h. The solution was evaporated under reduced pressure and the residue was dissolved in 3 N HCl and refluxed for 5 h. The hydrolysate was divided into two aliquots; one was assayed on a Beckman automatic amino acid analyser, the other treated with *n*-butanol-HCl and trifluoroacetic anhydride to convert amino acids to the N-trifluoroacetyl-*n*-butyl esters[10] for analysis by GC and GC–MS. Procedural blanks showed no amino acids.

Six amino acids (α-alanine, glycine, β-amino-*n*-butyric acid, β-alanine, N-methyl-β-alanine and aspartic acid) and one dicarboxylic acid (succinic acid) were found in the high temperature (1,060° C) run (Fig. 1), and their identities confirmed by comparison of their mass spectra with those of standard compounds[9,11]. The number of amino acids produced in this experiment is significantly less than the number reported before[1,3,4]. Also, in contrast to the predominance of the protein amino acids found in earlier studies, only three (glycine, α-alanine and aspartic acid) were identified. Our work seems to be unique in one other aspect. Reviewing the chemical evolution literature[12], Lemmon pointed out that in all simulated prebiotic experiments the yield of α-alanine was always higher than the yield of β-alanine. In our high temperature experiment, however, the yield of β-alanine (90 mol %) was approximately two orders of magnitude greater than the yield of α-alanine (1 mol %), and the yield of all compounds with β-separation of functional groups was much greater than those with α-separation of functional groups (Table 1).

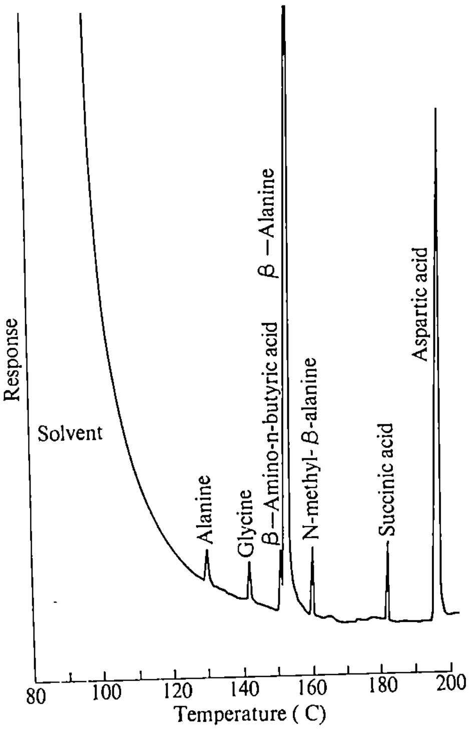

Fig. 1 Gas chromatogram of N-trifluoroacetyl-*n*-butyl esters of amino acids from the thermal synthesis at 1,060° C. Gas chromatography: 0.02 inch × 50 foot capillary column; OV17 coating; flame ionization detector; isothermal at 80° C for 4 min, then programmed to 200° C at 8° C min^{-1}.

Table 1 Compounds Produced by Thermal Synthesis

Compound	1,060° C (mol %)*	980° C (mol %)*	930° C (mol %)*
α-Alanine	1	12	4
Glycine	1	59	96
β-Alanine	90	28	—
N-Methyl-β-alanine	1.5	1	—
Succinic acid	1.5	—	—
β-Amino-*n*-butyric acid	1	—	—
Aspartic acid	3	—	—

* Yields were determined by amino acid analyser and gas chromatographic response.

The reactions and interactions of a series of intermediates (aminoacetonitrile, α-aminopropionitrile, hydrogen cyanide, methylamine, acrylonitrile, cyanoacetylene and crotononitrile) can be postulated to account for the products observed. Glycine and alanine would be the expected hydrolysis products of the corresponding nitriles (aminoacetonitrile and α-aminopropionitrile). β-Alanine has been identified in discharge experiments[13] and was postulated to be the hydrolysis product of β-aminopropionitrile, produced by Michael addition of ammonia to acrylonitrile. Also, acrylonitrile was predicted to produce succinic acid and N-methyl-β-alanine from hydrolysis of the nitrile intermediates originating from Michael addition of hydrogen cyanide and methylamine. Succinic acid was identified, but N-methyl-β-alanine, which is insensitive to the ninhydrin reaction commonly used in amino acid analysers, was not detected. In our synthesis at 1,060° C, we identified β-alanine, succinic acid and N-methyl-β-alanine, a finding consistent with the addition of ammonia, hydrogen cyanide and methylamine to acrylonitrile. The sequential addition of ammonia and hydrogen cyanide to cyanoacetylene is proposed as a route to a nitrile intermediate which is converted to aspartic acid after hydrolysis. Aspartic acid has been previously observed to be the product of such a reaction sequence[14]. The final compound identified, β-amino-*n*-butyric acid, would be the expected product obtained by hydrolysis of the nitrile intermediate resulting from addition of ammonia to crotononitrile.

The amino acids produced in lower temperature experiments are included in Table 1. At 980° C the concentration of β-alanine still exceeded the concentration of α-alanine; however, the α-amino acids were more abundant than the β-amino acids. Also, aspartic acid, β-amino-*n*-butyric acid and succinic acid were absent, indicating lack of the proposed intermediates hydrogen cyanide and crotononitrile. At this temperature, the ratio of glycine to α-alanine was approximately 5 to 1, whereas at 1,060° C the ratio was 1 to 1. At 930° C glycine and alanine were the only principal products, with the ratio of glycine to alanine nearly 20 to 1. These results suggest a temperature dependence for the proposed intermediates.

The yield from these experiments is low, typically 6–70 μmol of carbon incorporated into amino acids for each mole of methane used. This represents a yield of at best 0.007%, which is small compared with the 1.9% recently reported for a discharge experiment[8].

The unusual nature of the results presented here (predominance of β-amino acids at high temperature, limited number of amino acids produced, and low yield) presents problems in the interpretation of their relevance to chemical evolution. If these results are representative of what may have taken place on the primitive Earth, then the small yields of the thermal experiments are discouraging, particularly when compared with the discharge experiment which is postulated to have been a more abundant energy source for synthesis[15]. One way to circumvent this problem is to invoke a localized high concentration of thermally produced amino acids near a heat source such as a volcano. As vent temperatures are 1,050°–1,150° C[16], however, the products of such a synthesis would contain predominantly β-amino acids by analogy with the 1,060° C experiment. The formation of a primitive organism in an environment requiring the utilization of β-amino acids, followed by evolution into a modern organism that utilizes α-amino acids, is unattractive.

An alternative interpretation of our results is that they are not representative of what may have happened on the primitive Earth. If it is assumed that thermal synthesis was important for the prebiotic synthesis of amino acids, then future laboratory experiments should be designed to overcome the problems we encountered.

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Evidence for the Possible Immunogenicity of Δ^9-Tetrahydrocannabinol (THC) in Rodents

Chronic marihuana intoxication is accompanied by a marked pharmacological tolerance in all animal species[1–3], for example the necessity to increase dosage to obtain the effect of initial dosage. A similar degree of tolerance also develops to the effects of Δ^9-tetrahydrocannabinol (THC), the chief psychoactive substance of marihuana[4,5]. The time period required for the development of tolerance to THC in rodents[5] or dogs[6] is of the order of 5 to 9 d, which is similar to that required for the development of a cellular immune response. It was also shown by Agurell[7] that 3 d after the intravenous injection of tritiated Δ^9-THC there was a very high specific activity in the spleen. These observations led us to suggest[8] that tolerance development to marihuana and to Δ^9-THC might be mediated in part by the immune system, although no circulating antibodies to THC have ever been isolated.

Two series of experiments were performed to test this hypothesis. In the first we studied the effect of an immunosuppressant, azathioprine, on the development of tolerance to the hypotensive effects of THC in the spontaneous hypertensive rat (SHR). In the second we studied the effect of THC on blast transformation of lymphocytes sampled from mice sensitized to THC.

In the first series, three groups of twelve SHR rats weighing 234±2 g were studied. The two control groups were given daily either azathioprine (4 mg kg^{-1} i.p.) or THC by gastric tube (10 mg kg^{-1} in sesame oil). The third group was given 4 mg kg^{-1} azathioprine and 10 mg kg^{-1} THC. Treatment with azathioprine was started 2 d before administration of THC. Blood pressure was measured each day by tail plethysmography 24 h after administration of the drug.

Administration of azathioprine alone does not alter blood pressure. THC administration produces a significant hypotension for only 6 d, as previoulsy reported[5]. Pharmacological tolerance to this effect then follows. By contrast, the animals treated with THC and azathioprine do not develop tolerance to the hypotensive effects of THC during the experimental period of 13 d (Fig. 1). One may ask whether the immuno-

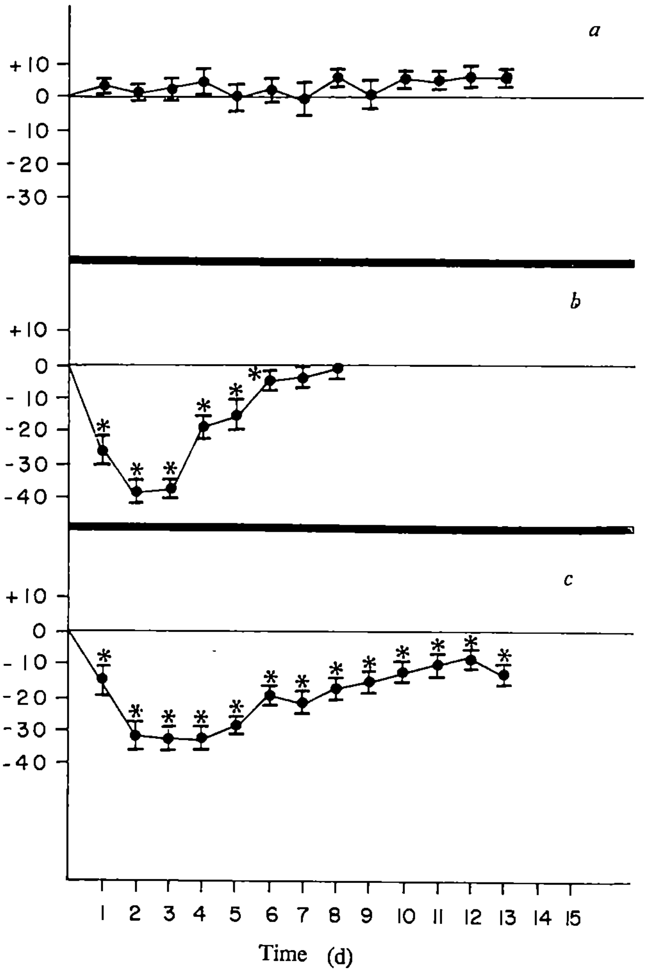

Fig. 1 Effects of an immunosuppressant, azathioprine, on the development of the hypotensive effects of Δ^9-THC in the spontaneously hypertensive rat (SHR). Twelve animals were used in each group. *a*, Azathioprine, 4 mg kg^{-1} d^{-1}; mean control blood pressure 195±3 mm Hg; *b*, THC, 10 mg kg^{-1} d^{-1}; mean control blood pressure 199±3 mm Hg; *c*, azathioprine, 4 mg kg^{-1} d^{-1}+THC, 10 mg kg^{-1} d^{-1}.

*Significantly different from control blood pressure changes ($P<0.05$).

suppressant therapy inhibited the development of pharmacological tolerance to THC.

In the second series, three groups of five male mice of the CD1 strain were used. The two experimental groups were given, at one week intervals, two intravenous injections of THC in alcohol (5 mg kg^{-1}) and one intraperitoneal injection (10 mg kg^{-1}) in Freund adjuvant. The control group was given similar injections of the same volume of vehicle. All animals were killed 24 to 48 h after the last injection.

The spleens of five animals were pooled and sectioned in fine fragments in culture medium RPMI 640, *p*H 7.2. The cellular suspension thus obtained was filtered on a grid of mesh 60, washed and adjusted so that the cell concentration was 3.5×10^6 cells ml^{-1}.

Because of the toxicity of alcohol to lymphocytes, THC was added to the cell culture after preparation as follows[9]. The alcohol of the THC solution was evaporated by bubbling nitrogen. The resinous residue was then resuspended by magnetic stirring for 12 h at 4° C in a solution of foetal calf serum containing 50 mg of bovine serum albumin (BSA) ml^{-1}. (In such a preparation, 85% of the THC is recovered in the BSA solution[9].) THC in BSA and foetal calf serum was added to the samples so that the final concentration of THC was 5 mg l^{-1} (1.6×10^{-5} M). THC vehicle was added to the control samples, and in one group of controls phytohaemagglutinin P (PHA P, Difco), suspended in sterile water, was added at 10 µl per tube. After 48 h of incubation at 37° C, 2.5 µCi of ^{3}H-thymidine with a specific activity of 1.9 Ci mmol^{-1} was added to the samples for the final 24 h of culture.

At 72 h the tubes were placed on ice. After two washings, in RPMI, cells were spread on glass slides, fixed in methanol, and dipped in an Ilford emulsion. After 30 h exposure they were developed and stained with Wright Giemsa; those cells which had incorporated ^{3}H-thymidine were counted. The results are given as the percentage of cells in the population scored as lymphoblasts (1,000 to 1,500 cells were counted in each experiment). Adler *et al.*[10] have reported that the time course of lymphoblastic transformation appreciated by morphological criteria as described by Chessin *et al.*[11] was paralleled roughly by incorporation of ^{3}H-thymidine, in both stimulated and unstimulated cells. The autoradiographic technique which we used is more accurate as only cells which have incorporated ^{3}H-thymidine are counted.

Table 1 indicates that THC, unlike other substances of vegetable origin such as phytohaemagglutinin (PHA), does not stimulate mitosis of lymphocytes in culture. Furthermore, the lymphocytes of mice treated with THC and incubated in the presence of the drug incorporate ^{3}H-thymidine at a faster rate, as indicated by the increased blast transformation. The specific blastogenic activity of THC is similar to that induced by other antigenic substances. It is observed only on spleen cells sampled from animals sensitized to the same antigen as the one which is introduced into the culture.

The blast transformation we have observed with THC is similar in extent to that observed with other antigens, but is much less than that stimulated by PHA. These experiments add further weight to the hypothesis we suggested previously[8]. The inhibition of tolerance development in animals treated with azathioprine could be related to an inhibition of the immune response.

Table 1 Percentage Blast Transformation of CD1 Spleen Cells Observed after ^{3}H-Thymidine Incorporation. Cells from Five Pooled Spleens were Used in each Experiment

	Control mice	Mice treated with THC	
		Exp. I	Exp. II
Lymphocytes in culture without THC	4.7%	7.2%	6.8%
Lymphocytes in culture with THC	4.3%	13.5%	13.1%
Lymphocytes in culture with PHA	37.8%	—	—

That a small molecule like THC (molecular weight 314.5) could induce a cellular immune response usually produced by macromolecules seems at first unlikely. But THC, which is insoluble in water, is transported in the plasma, 90% bound to protein[12]. It is conceivable that THC might constitute a hapten *in vivo* which could trigger an immune response, as indicated by blast transformation. There is a similar immune response towards morphine in mice[13].

If a similar immune response to THC occurs in man, it could account for the eosinophilia[14] and the allergic responses which have been observed among cannabis users[15,16]. It could also account for the extreme variability of individual reactions reported after usage of this drug[17].

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Production of Antibody to Sheep Red Blood Cells by Human Tonsil Cells *in vitro*

Two types of lymphocyte cooperate in initiating the humoral response to antigen: the B cell is the progenitor of antibody-forming cells and the T cell acts in a helper capacity. The activity of a third cell, the macrophage, is also required. Although the functions of B and T cells are antigen-specific, that of the macrophage is not. Most knowledge about the kinetics and distribution of the two types of lymphocytes, their specificity, and their susceptibility to inhibition or stimulation, has been established by experiments with mice. Progress has been facili-

Table 1 Lack of Production of Antibody to SRBC by Cultures of Human Blood Lymphocytes and Tonsil Cells from Individual Donors

Cultured cells	PFC* on day 5 with SRBC†	PFC* on day 5 without SRBC
Blood	0	0
Blood	0	0
Blood	0	0
Tonsil	42	68
Tonsil	3	6
Tonsil	0	0

* Plaque-forming cells per culture.
† 3×10^5 SRBC per culture.

tated in particular by the development of methods that measure the response of mouse spleen cells to antigen *in vitro*; we report here some experiments with human lymphocytes from blood and tonsils, the two sources of such cells most readily available.

We used the culture method described by Mishell and Dutton[1] and the ficoll-hypaque method for purifying blood lymphocytes described by Boyum[2]. The tonsils were cut in half with a hot scalpel blade, and cells were obtained from the cut surface by mechanical dissociation.

Culture with sheep red blood cells (SRBC) did not induce blood lymphocytes or tonsil cells from individual donors to produce antibody to SRBC (Table 1). This was taken to indicate that one or more of the cellular components (T cells, B cells or macrophages) did not function properly or were not present in adequate numbers. To compensate for a possible lack of macrophages in the tonsil cell preparations, we added increasing numbers of blood leukocytes (known to be rich in phagocytic cells) to the tonsil cells. Table 2 shows that these mixed cultures produced antibody to SRBC in the presence of SRBC. Because the blood cells and tonsil cells used in these experiments came from different donors, the results could be interpreted in two ways: (1) the addition of blood monocytes did in fact compensate for a deficiency in phagocytes of the tonsil preparation, or (2) the response was a consequence of stimulation by foreign alloantigens, which can amplify or replace the specific helper function of T cells in the mouse[3,4].

Table 2 Production of Antibody to SRBC by Mixed Cultures of Tonsil Cells and Blood Lymphocytes

Tonsil cells	Blood lymphocytes	PFC Day 4	PFC Day 5	PFC Day 6
5×10^6	—	13	22	25
—	5×10^6	—	3	16
5×10^6	10^6	105	286	380
5×10^6	3×10^5	77	122	175
5×10^6	10^5	18	76	41
5×10^6	3×10^4	6	17	5

Subsequent experiments favoured the latter explanation. (*a*) Tonsil cells that did not produce antibody to SRBC, when cultured individually, did so in mixed culture (Table 3), showing that human tonsils do contain B cells able to respond to SRBC; the same cannot be said of blood lymphocytes because they did not produce antibody to SRBC even in mixed cultures. (*b*) The positive response of mixed tonsil cultures, differing only in alloantigens, as opposed to individual cultures, suggests that tonsils contain sufficient macrophages. This is also indicated by the fact that neither mouse macrophages nor 2-mercaptoethanol[5] induced antibody production to SRBC in individual tonsil cultures.

Thus T cell deficiency rather than macrophage deficiency seems to be the limiting element in the tonsil cell population. Because of a recent report that bacterial lipopolysaccharides can substitute for T cell function in mice[6] we then investigated the effect of *E. coli* endotoxin on the production of antibody to SRBC by human tonsil cells. As Table 4 shows, the addition of *E. coli* lipopolysaccharide to cultures of individual tonsils induced the production of antibody to SRBC, and the response was of the same magnitude as that seen in mixed cultures. We could also elicit the production of hapten-specific antibody to 2,4,6-trinitrophenol, conjugated with SRBC, by adding endotoxin to cultures of tonsil cells. The latter finding offers a means of investigating the function of T and B cells independently, using the method developed for the mouse[7]. So far, our experiments have shown differences in responsiveness of individual tonsils depending, for example, on the age of the donor and on the size of the tonsil.

Table 3 Production of Antibody to SRBC by Mixed Tonsil Cell Cultures

Source of tonsil cells	PFC on day 5 with SRBC†	PFC on day 5 without SRBC
A*	32	30
B	6	8
C	14	18
A+B	170	80
A+C	88	6
B+C	200	48
D	8	4
E	76	18
F	104	100
D+E	100	44
D+F	600	208
E+F	520	144

* 10^7 cells per culture; 5×10^6 cells from either source in mixed cultures. The letters designate tonsils from different subjects.
† 3×10^5 per culture.

Table 4 Effect of *E. coli* Lipopolysaccharide on the Production of Antibody to SRBC by Cultured Tonsil Cells

Source of tonsil cells*	*E. coli* lipopolysaccharide†	PFC on day 4 with SRBC‡	PFC on day 4 without SRBC
G	–	116	8
	+	590	28
H	–	102	100
	+	1,260	280
I	–	65	110
	+	440	170
K	–	6	6
	+	97	26
L	–	0	0
	+	10	0
M	–	24	12
	+	52	14

* 10^7 per culture; the letters designate tonsils from different subjects.
† 0.030 mg per culture.
‡ 3×10^5 per culture.

The background of the plaque-forming cells in mixed tonsil cultures, and in cultures of individual tonsils to which *E. coli* lipopolysaccharide had been added, is not at present explained. It may indicate pre-existing sensitization to cross-reacting sheep antigens, red cell antigens or bacterial antigens or, in the case of *E. coli* lipopolysaccharide, cross-reactivity between this material and SRBC antigens.

In vitro studies of this sort may lead (as they have done in the mouse) to better understanding of factors that govern the immune response in man, and may suggest ways of manipulating them for the purpose of correcting immunological defects.

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Basis of the Defect in α-1-Antitrypsin Deficiency

DEFICIENCY of the serum protein α-1-antitrypsin is an important cause of obstructive lung disease in adults and of hepatitis in children. The deficiency state can be considered as one variant form of α-1-antitrypsin but several other variants are also known, occurring with a frequency of approximately three per hundred in Northern Europeans[1]. For the most part these are simple electrophoretic variants which are functionally normal and explicable as simple amino acid substitutions. The proportion of the variant form, and family studies, strongly support a single autosomal locus for α-1-antitrypsin.

There are, however, some perplexing observations. First, whereas electrophoresis of α-1-antitrypsin in agar gel (*p*H 8.6) gives a single band, electrophoresis in borate starch gel at *p*H 4.9 gives a complex multiband pattern (Fig. 3*a*). This pattern has been well studied and is used by Fagerhol and Laurell[2] in their Pi system, which provides a nomenclature for classifying the variant genes (for example *M* for the normal gene, *Z* for the deficient gene). It has been difficult, however, to explain fully these complex patterns in terms of a single locus and they could be interpreted as evidence of considerable genetic heterogeneity. Another perplexing feature is seen in the homozygous deficiency state (*ZZ*) in that the deficiency is never complete, some 10% of the α-1-antitrypsin always being present in the serum. Furthermore, this remnant α-1-antitrypsin has a distinctly slower mobility than normal on agar gel electrophoresis. In acid starch gel electrophoresis the *ZZ* pattern (Fig. 3*b*) differs even more markedly in both distribution and mobility from the *MM* pattern. Together these findings make it difficult to explain the deficiency state on the basis of a simple genetic lesion.

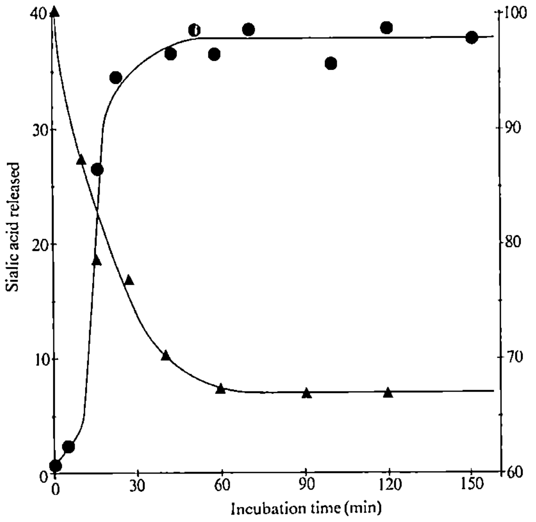

Fig. 1 Incubation of α-1-antitrypsin with neuraminidase gave a decrease in electrophoretic mobility concomitant with the release of sialic acid (N-acetylneuraminic acid). The mobility change (▲; % of normal) was determined using immunoelectrophoresis in agar gel (see Fig. 2). The sialic acid release (●) is given in μmol × 27 × 10^3 per ml of extract.

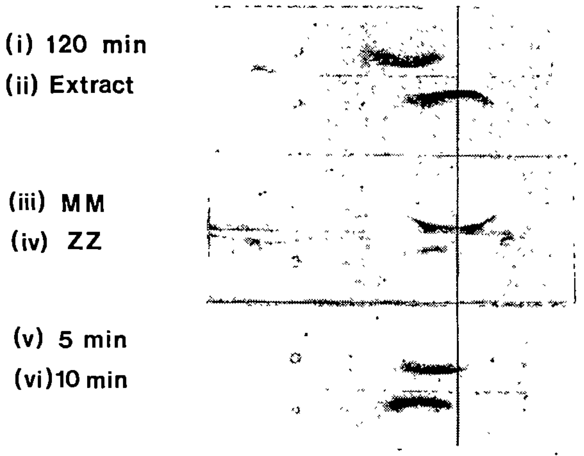

Fig. 2 Immunoelectrophoresis (agar, *p*H 8.6) against specific α-1-antitrypsin antiserum. The prepared extract (ii) shows the normal *MM* mobility as indicated by the drawn line. Incubation of the extract with neuraminidase shows a progressive decrease in mobility (V, VI, I), the 5 min incubation sample (V) having a 10% decrease in mobility equivalent to that seen in the deficiency *ZZ* state (IV).

We believe that the electrophoretic findings can be explained in terms of the carbohydrate moiety of α-1-antitrypsin and that a lesion in the structure of this would explain both electrophoretic and morphological findings in the homozygous deficiency state.

The electroheterogeneity of α-1-antitrypsin is not contradictory of a single locus and has been similarly observed with other glycoproteins (for example porcine RNase). It is possible that the banding represents microheterogeneity of the carbohydrate though the requirement of borate in the α-1-antitrypsin system suggests a complexing that may vary with ordered structure. Supporting evidence that the banding reflects variations in ordered structure is our finding that addition of 6 M urea to the borate acid starch gel system results in a single band of α-1-antitrypsin.

We also found that the appearance of the *ZZ* antitrypsin could be duplicated by partial removal of sialic acid (N-acetylneuraminic acid) from the normal α-1-antitrypsin. Human α-1-antitrypsin was separated from other glycoproteins by preparative electrophoresis in polyvinyl chloride/acetate. The eluted and concentrated material was then subjected to timed digestion with neuraminidase (Type VI, Sigma) and measurements made of sialic acid release[4] and of changes in the electrophoretic mobility of the α-1-antitrypsin in agar gel at *p*H 8.6. As shown in Fig. 1 the mobility decreased concomitantly with the release of sialic acid, the final mobility being approximately 70% of that of the normal. As Fig. 2 shows, the *ZZ* antitrypsin has a 10% decrease in mobility equal to that after incubation for 5 min in the system used and equivalent to one third the decrease of the complete reaction. Incubation of whole human serum was then performed using neuraminidase concentrations calculated to give appropriate partial digestion. Electrophoresis confirmed that there was again a slowing of α-1-antitrypsin equivalent to the *ZZ* form. More significantly, electrophoresis in acid starch gel gave a striking reproduction of the *ZZ* pattern both in the mobility and proportion of peaks (Fig. 3*c*). Both the *ZZ* antitrypsin, and the normal treated with neuraminidase, retain their antitryptic activity[5].

Thus the changed appearance of α-1-antitrypsin in the deficiency state can be explained by a partial loss of sialic acid. This fits in well, together with other observed changes which have been reviewed by Sharp[6] to give a coherent hypothesis for the disease process. The carbohydrate moiety of glycoproteins

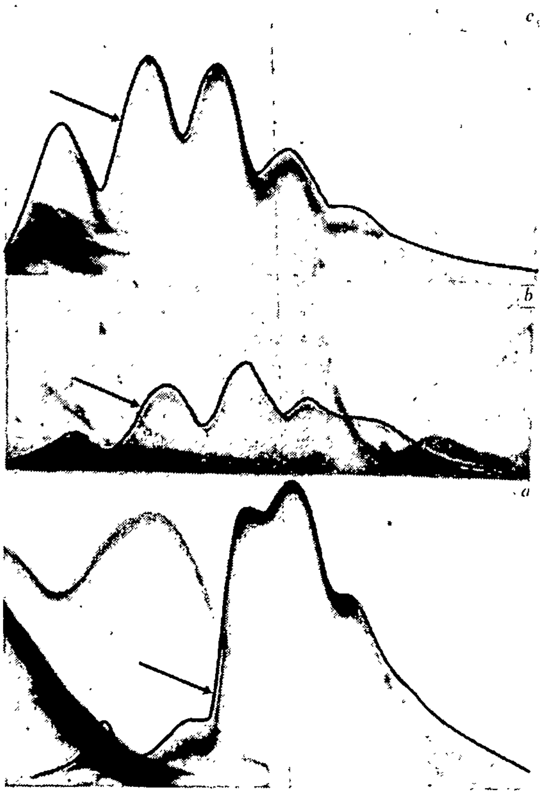

Fig. 3 Two dimensional immunoelectrophoresis of serum. First dimension starch gel *p*H 4.9, second dimension agar containing polyvalent antisera. The three samples have been run under the same conditions and aligned in terms of the buffer front and origin. The α-1-antitrypsin line is arrowed and outlined. (*a*), Normal (*MM*) pattern; (*b*), concentrated homozygous deficient (*ZZ*) serum showing characteristically diminished peaks of slower mobility; (*c*), normal serum incubated with neuraminidase. The *ZZ* distribution is closely reproduced.

is almost certainly of importance for their active transport across the cell membrane. We propose that in the deficiency state there is incomplete addition of sialic acid to the molecule, which can be expected to accumulate intracellularly with only small amounts escaping by passive diffusion. In fact, α-1-antitrypsin in the deficiency state accumulates in large amounts at the site of synthesis in the liver cells. In particular it is associated with the rough endoplasmic reticulum which is believed to be a site of synthesis of the carbohydrate portion of glycoproteins.

The accumulation within the liver cell renders it particularly susceptible to hepatitis and is thought to be the major factor in the development of the associated childhood cirrhosis. Small amounts of antitrypsin may be released by passive diffusion to give the circulating *ZZ* antitrypsin but overall levels are presumably insufficient to protect lung tissue from proteolysis.

It is therefore our view that the deficiency state for α-1-antitrypsin can be explained on the basis of a single locus of origin, the defect being in the synthesis of the carbohydrate moiety resulting in defective release from liver cells.

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Glycine N-Methyltransferase is a Regulatory Enzyme which Increases in Ageing Animals

Transfer RNAs are modified extensively after their primary synthesis is completed. Such modifications, which are necessary for the proper functioning of tRNA in protein synthesis[1-3] and transcriptional control[4], are achieved by enzymes which include the tRNA methyltransferases[5]. In certain adult organs the tRNA methyltransferases are under the influence of a competing methylating enzyme, glycine N-methyltransferase[6]. This enzyme system, which is present in adult liver, kidney and pancreas (but not in the corresponding foetal organs or in tumour tissues), not only competes for the methyl donor, S-adenosylmethionine, but is much less sensitive to inhibition by the product of the reaction, S-adenosylhomocysteine, than are the tRNA methyltransferases. Thus, there is a dual mechanism of control.

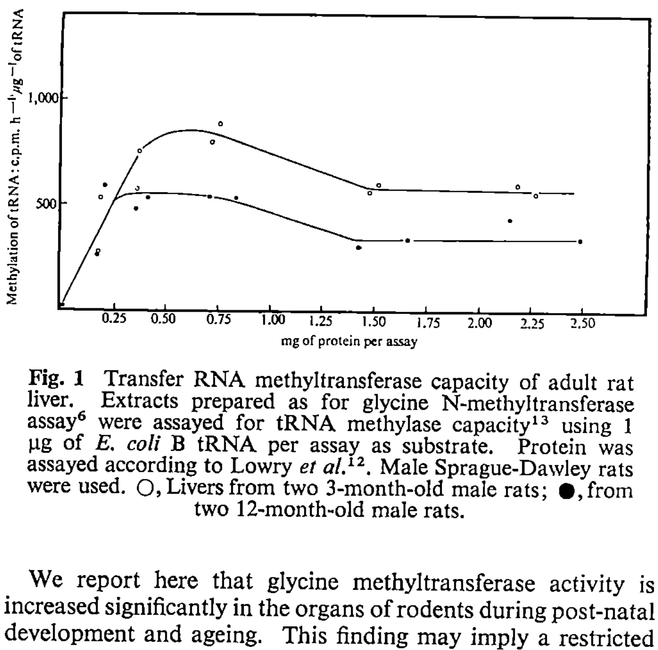

Fig. 1 Transfer RNA methyltransferase capacity of adult rat liver. Extracts prepared as for glycine N-methyltransferase assay[6] were assayed for tRNA methylase capacity[13] using 1 µg of *E. coli* B tRNA per assay as substrate. Protein was assayed according to Lowry *et al.*[12]. Male Sprague-Dawley rats were used. ○, Livers from two 3-month-old male rats; ●, from two 12-month-old male rats.

We report here that glycine methyltransferase activity is increased significantly in the organs of rodents during post-natal development and ageing. This finding may imply a restricted activity of the tRNA methyltransferases in ageing animals with a possible consequent deficiency of modification of the tRNA populations.

The increase in glycine N-methyltransferase activity is not merely a property of the transition from foetal to adult organism. As Table 1 shows, the specific activity of the glycine N-methyltransferase in rat liver increased 30% during the period between 3 and 12 months, while in rat kidney the increase was 40%.

The effect of these increases in glycine N-methyltransferase activity on the methyltransferase capacity of 3 months (post-pubertal) and 12 months (mature) rat liver and kidney *in vitro* can be seen in Figs. 1 and 2. These assays are performed using crude, undialysed 100,000*g* supernatant extracts of the organs. Thus, glycine is present as well as glycine N-methyltransferase. At low protein concentrations, where the competing enzyme system is diluted out, the specific activities of the tRNA

Table 1 Specific Activity of Glycine in N-Methyltransferase in Liver and Kidney of Post-pubertal and Mature Rats

Organ	Post-pubertal 3 Months	Mature 12 Months
Liver	22.6	31.0
Kidney	3.6	5.8

Specific activity is expressed as nmol sarcosine formed per mg protein per 30 min. Extracts were prepared and assayed for activity according to Kerr[6], except that $^{14}CH_3$-S-adenosylmethionine (specific activity 53 Ci mol^{-1}) was present at a concentration of 2×10^{-5} M. Protein was assayed according to Lowry *et al.*[12] Rats were male Sprague-Dawley animals.

Age	No. of animals	Body weight Average (g)	Range (g)
3 months	4	267	235–309
12 months	8	429	391–471

methyltransferases from 3 and 12-month-old rats seem to be the same. At the higher protein concentrations required to reach saturation capacity, however (the maximum number of methyl groups which the enzymes from a given source can introduce into a unit amount of heterologous tRNA), there is a marked difference in the saturation levels reached by the tRNA methyltransferases of organs from 3 and 12-month-old rats. The total capacity for tRNA methylation is lowered in the older animals by about 35%.

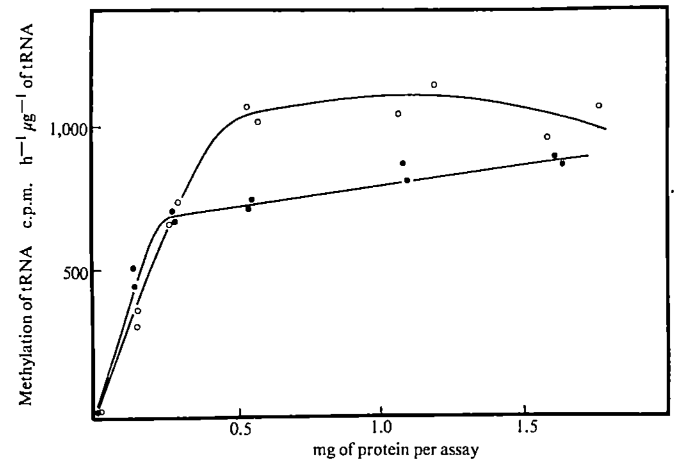

Fig. 2 Transfer RNA methyltransferase capacity of adult rat kidney. Experimental details were as for Fig. 1. ○, Kidneys from two 3-month-old male rats; ●, from two 12-month-old male rats.

A similar increase in glycine N-methyltransferase specific activity was observed in the livers of C57Bl/6J male mice. Between 12 months (mature) and 30 months (senescent), which is the average life span[7], the specific activity of the enzyme undergoes a linear increase (Fig. 3). The specific activity of tRNA methyltransferase of mouse livers is also diminished with age (Fig. 4).

These measurements demonstrate that glycine N-methyltransferase increases during ageing as well as during maturation. If the concomitant decrease in activity of tRNA methyltransferase *in vitro* is an accurate reflexion of the activity of these enzymes *in vivo* then the tRNA of ageing animals may become progressively hypomethylated with a possible decrease in functional fidelity. Changes in tRNAs and their modification have been reported in two different ageing biological systems.

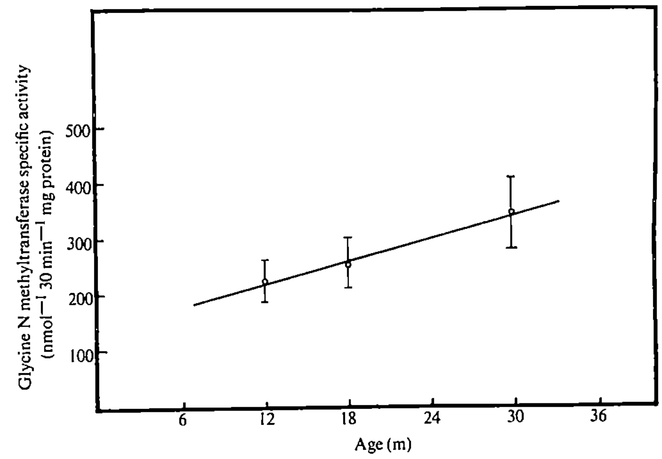

Fig. 3 Glycine N-methyltransferase activity of adult C57Bl/6J male mouse liver. Livers were frozen in dry ice immediately after death. They were subsequently held at −80° C until use. Preparations and assays were as described in Table 1 except that S-adenosylmethionine was used at 5×10^{-5} M in all reactions. Each mean is from two determinations on each of five animals. Careful husbandry procedures and observations at necropsy[7,16] eliminated any mice with gross pathological lesions, particularly the reticulum cell sarcoma which is characteristic of senescence in this strain[7]. ○, Mean (livers) from five mice; I, standard deviation.

Differences in tRNA from aged compared with young mosquitoes have been reported[8], and in the apical part of the wheat-leaf, which has stopped growing, there is a tRNA^{Phe} which is improperly modified and consequently its attachment to ribosomes is impaired[9]. A hypothesis of random accumulation of errors in proteins synthesized, as being instrumental in the ageing process, has been previously proposed[10] and the accumulation of altered proteins in ageing human fibroblasts in tissue culture has been recently demonstrated[11].

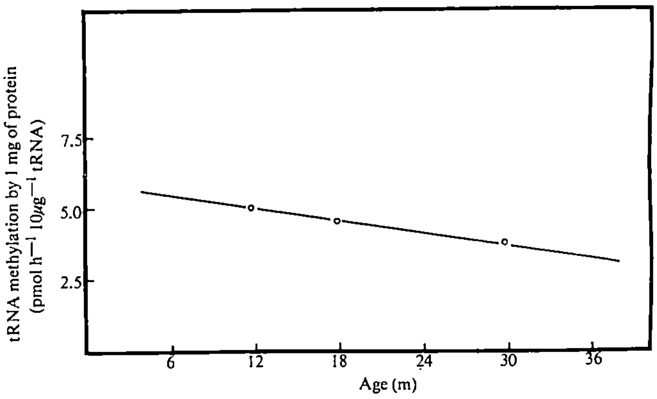

Fig. 4 Transfer RNA methyltransferase activity in C57Bl/6J male mouse livers. Liver treatment was the same as for Fig. 3. The tRNA methyltransferase activity assay was as for Fig. 1 except with 10 μg of *E. coli* B tRNA per assay. Protein was assayed according to Lowry *et al.*[12].

Increased glycine N-methyltransferase activity and putatively consequent hypomethylation during post-natal development and ageing could result in the appearance of novel isoaccepting tRNA species with ensuing effects on protein synthesis. A novel serine tRNA is found, for example, in the uterus of ovariectomized rats, whose tRNA methyltransferase activity is diminished[14]. Because of the sensitivity of tRNA methylation to hormones, it is possible that the increased glycine N-methyltransferase activity represents the response of the liver and kidney to changes in hormones related to age, as has been shown for certain other age-related changes of enzyme activities[15,16].

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Prenatal Hypothyroidism and Brain Composition in a Primate

THE neonatal rat has been used almost exclusively as a convenient model for elucidating the effects of thyroid hormone or thyroid ablation on the developing central nervous system[1-4].

Although valuable information has been obtained, the growth and development of rats differs from that of primates[5,6]. Recent studies[7-10] have shown that the monkey (*Macaca mulatta*) is a more suitable model for the evaluation of experimental situations applicable to the human foetus. Six pregnant rhesus monkeys were injected with 2 mCi Na^{131}I kg^{-1} intravenously at 71–88 d of gestation. Casearean section was performed on these and an equal number of untreated pregnant females at 150 d (full term gestation is 165±4 d).

Foetal and maternal plasma thyroid hormone levels were indicative of hypothyroidism in the ^{131}I-treated animals (Table 1) although the mothers showed no apparent clinical symptoms during pregnancy. The treated foetuses weighed somewhat less than expected for gestational age and showed reduced respiratory movements and cretinoid facies. The thyroid gland was completely absent. The weight of the whole brain (including its parts) was within normal limits but the pituitary was heavier than normal. Details on physical growth and skeletal maturation including a discussion of placental thyroid hormone transfer have been published elsewhere[11].

Table 1 Thyroid Hormone in Foetal and Maternal Plasma

	Maternal	Foetal
Protein-bound iodine (μg%)		
Control	7.2±1.2	7.3±0.5
^{131}I	4.6±1.4	4.7±0.4
Thyroxine (μg%)		
Control	9.6±2.3	7.2±2.1
^{131}I	2.3±0.9	0.6±0.8

Cerebrum and cerebellum were analysed separately for DNA[12], RNA[13], protein[14], Na$^+$+K$^+$-ATPase[15,16] and carbonic anhydrase activity[17], cholesterol[18,19], lipid N-acetylneuraminic acid (lipid NANA)[18,20] and chloride[21].

Water was determined by desiccation and partitioned into apparent extracellular and intracellular compartments assuming that the chloride space is a valid measure in this situation. Stereological measurement of the extracellular space of developing rat cortex following a freeze-substitution technique[22], which agrees with chloride space determinations in similar samples[23], adds credence to this approach.

The data are expressed in terms of total amount and are presented as percentages of the normal control values. In Fig. 1, the cerebrum had essentially the same mean weight, water and DNA content (cell population) as the normal controls. Total protein and non-protein dry solids (lipid, polysaccharides and mineral) were significantly reduced. The non-chloride space (an index of neuronal volume)[23] was significantly reduced while the concomitant increase in chloride space was not significantly different. The cerebellum showed a similar but more marked pattern with highly significant reductions in non-chloride space (21%), protein (23%) and non-protein dry solid (15%).

Fig. 2 documents the changes in RNA, two neuronal components (lipid NANA and total N$^+$+K$^+$-ATPase activity) and two extraneuronal components (cholesterol and total carbonic anhydrase activity). Changes in weight, DNA and protein are reproduced for comparison. RNA was significantly reduced in the cerebrum (17%) and cerebellum (19%). Cerebral neuronal components—lipid NANA and Na$^+$+K$^+$-ATPase—were deficient by 17% and 34% respectively, and in the cerebellum both these components were reduced 30%. Cerebral cholesterol and carbonic anhydrase activity were reduced 16% and 43% respectively. In the cerebellum a greater reduction of these components was observed. The cholesterol content was reduced 28% and the total carbonic anhydrase activity was reduced 48% by comparison with control values.

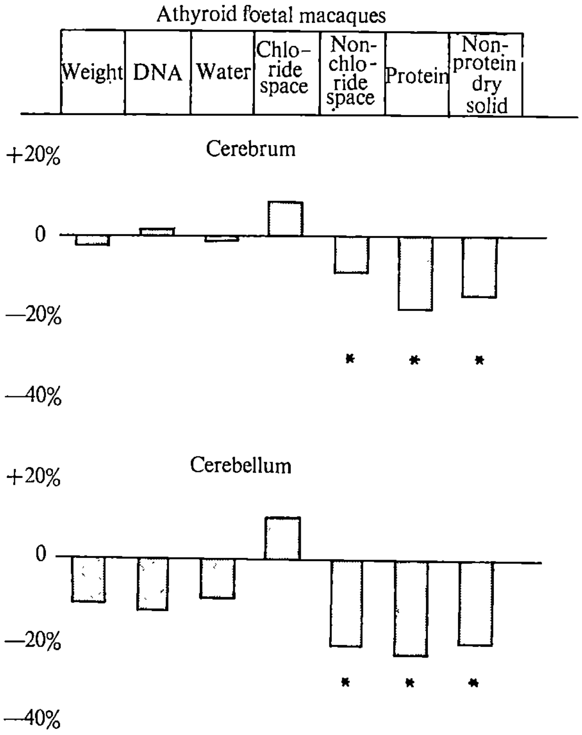

Fig. 1 Changes in the gross composition of the cerebrum and cerebellum of athyroid foetal macaques. Each component is represented as the mean percentage change relative to the control (zero line). Weight and DNA are included for reference. The asterisk indicates statistical significance. $P<0.01$.

Many of the changes described here have been previously

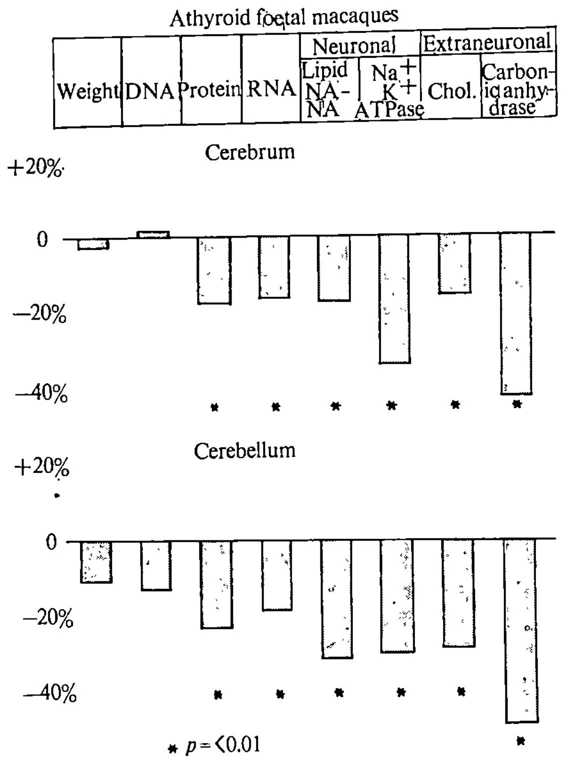

Fig. 2 Changes in the content of RNA, neuronal and extraneuronal components of the cerebrum and cerebellum of athyroid foetal macaques. Weight, DNA, and protein are illustrated for reference. The data are presented in the same way as in Fig. 1.

observed in young rats receiving thyroid ablation at birth[1–4,24]. Data describing the chemical composition of the brain in this rat extrauterine model include normal DNA contents with absolute reductions in RNA, protein and cholesterol. Elevation in the percentage of water and changes in chloride concentration have also been described. Reduction in $Na^+ + K^+$-ATPase activity has been consistently demonstrated[25,26]. This depression, when expressed as activity per gramme was greater for cerebral cortex than for the cerebellum. A similar pattern is formed in the foetal macaque brain in the absence of thyroid function. It is emphasized that increase in DNA concentration in the cerebrum and reduction in the cerebral weight, which occur in the neonatal hypothyroid rat, were not observed in this study.

Carbonic anhydrase, an enzyme found exclusively in glia[27], is greatly reduced in the brains of our athyroid foetuses. This enzyme has not been determined in the hypothyroid rat brain. Cholinesterase activity (which predominates in glial cells) has, however, been assayed and found to be decreased[28].

Gangliosides (lipid NANA) are concentrated in the grey matter of the central nervous system and are associated with maintenance of electrical activity and ion transport[29]. Subcellular studies show gangliosides in the microsomal fraction, presumably with fragments of dendrites and nerve endings[29,30]. $Na^+ + K^+$-ATPase has a similar distribution[29–32] and is closely associated with the appearance of electrical phenomena in the cerebrum[15].

Brain $Na^+ + K^+$-ATPase activity is depressed in hypothyroidism in rats and primates while a reduction in ganglioside content has only been observed in this study. The report of a normal content of ganglioside in the hypothyroid rat cerebrum is not explained considering that the same workers described reduced dendritic arborization[33] and evidence for a reduction of synaptic terminals[1,34].

Examination of hypothyroid rat cerebral cortex by interference microscopy and planimetry reveals significant reduction in the dry mass and area of cortical neurones[34]. This agrees with our findings of a reduced non-chloride space.

As anorexia is a common accompaniment of hypothyroidism and maternal malnutrition may cause changes in foetal brain growth, it can be asked whether the changes observed in this study are an effect of undernutrition. Female rhesus monkeys have been subjected to severe protein/calorie restriction during pregnancy. Preliminary results indicate that the cerebrum of their offspring is unaffected while minimal changes concerning myelination are seen in the cerebellum. Legrand's[35] histological studies of underfed and hypothyroid rat cerebellum suggest that abnormalities observed after neonatal hypothyroidism are specific for that condition.

From our results, one can conclude that overall RNA and protein synthesis is depressed in the foetal brain in the absence of thyroid hormone. Our data indicate that both neuronal and neuroglial cell populations are functionally affected in cerebrum and cerebellum. Further work with this primate model should afford a better understanding of the role of foetal thyroid hormone in the development of the brain.

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Identification of a Possible Subunit of Hexosaminidase A and B

THE two chief forms of N-acetyl-β-D-hexosaminidase of human tissues are distinguishable by their electrophoretic behaviour and heat stability and can be separated by ion exchange chromatography[1,2]. These properties have been used in a differential assay of the forms for diagnostic purposes in cases of lysosomal storage disease. Tay-Sachs' disease is characterized by the lack of the thermolabile hexosaminidase A and by elevated amounts of the more stable hexosaminidase B[3] and another form of the enzyme as yet incompletely characterized. In Sandhoff's disease[4] which resembles Tay-Sachs' disease in the resultant accumulation of cerebral ganglioside GM_2, all forms of the enzyme are at a very low concentration. The A and B forms of the enzyme are closely related at a molecular level and antisera raised to either enzyme separated from human liver[5] or from human placenta[6] cross-react with the other enzymic form.

In our earlier reports we noted that hexosaminidase A could be converted to a form resembling but not necessarily identical to hexosaminidase B, by enzymic removal of sialic acid residues. This has led some authors[7,8] to suggest that hexosaminidase B is a precursor of A and Tay-Sachs' disease represents a genetic lack of a specific sialo-transferase. No such transferase has yet been demonstrated, however, and the theory does not explain the spontaneous origin of new forms that arise during controlled denaturation[9].

In order to explain these findings we proposed that the enzymes contain at least two different subunits[10], one of which carries the active site for hydrolysis and is largely responsible for the observed antigenicity, being common to all forms of the enzyme, and the other of which is a "specifier" subunit characteristic of hexosaminidase A and conferring on the active site the specific ability to hydrolyse ganglioside GM_2. Tay-Sachs' disease would then represent a mutation of the specifier subunit, leaving hexosaminidase B unaffected, whereas Sandhoff's disease would result from loss of activity of the common enzymic subunit.

This biochemical model has been supported and reiterated recently by Beutler's report[11] of the presence of non-enzymic cross-reacting material, corresponding to forms A and B in liver from a patient with Sandhoff's disease. No cross-reacting material corresponding to the missing hexosaminidase A was found in Tay-Sachs' liver.

We have now detected and partially characterized a protein of low molecular weight, present in normal liver and in livers from patients with Sandhoff's disease and Tay-Sachs' disease, that has several of the expected characteristics of the proposed enzymic subunit.

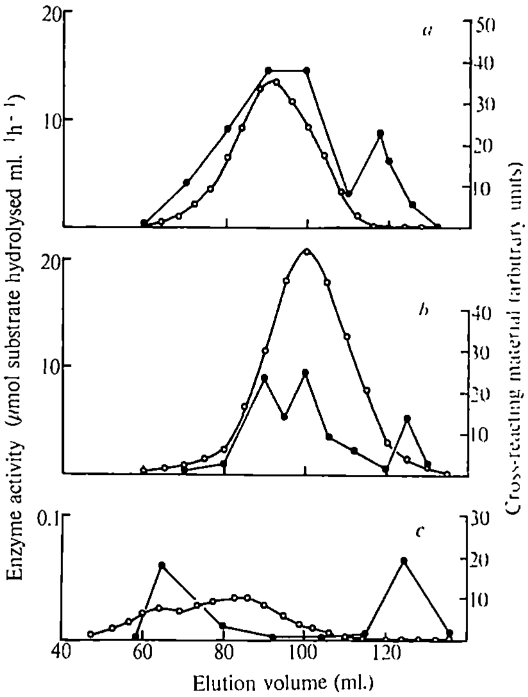

Fig. 1 Separation of hexosaminidases and cross-reacting material to antihexosaminidase A serum on 'Sephadex G-150'. *a*, Normal human liver; *b*, Tay Sachs' diseased liver; *c*, Sandhoff's diseased liver. - - -, Hexosaminidase; —, cross-reacting material.

Samples of Sandhoff, Tay-Sachs', and control livers were separated by gel permeation chromatography on columns of 'Sephadex' G-150, and the eluate tested for hexosaminidase activity, using the synthetic substrate 4-methylumbelliferyl-N-acetyl-β-glucosaminide, and for cross-reactivity to an antihexosaminidase A serum. Details of the preparation of the antigen for this antiserum have already been published, and it was shown to be devoid of hexosaminidase B and any other proteins of molecular weight lower than 10 (ref. 5).

It was found (Fig. 1) that the cross-reactivity occurred not only in those samples containing active hexosaminidase (molecular weight 130,000) but also in a component of low molecular weight, estimated to be 20,000–25,000 by comparison with marker proteins of known size. Sandhoff and Tay-Sachs' tissue also showed not only macromolecular cross-reacting material corresponding in size to the complete enzyme but considerable amounts of the low molecular weight material which was entirely devoid of hydrolytic activity towards the synthetic substrate. The macromolecular cross-reacting material of Sandhoff tissue cochromatographs with the low but measurable amount of activity present in these cases, whereas in Tay-Sachs' tissues it cannot be distinguished from the considerable amount of remaining hexosaminidase.

The material of low molecular weight, which is being further purified and characterized, has thus the following properties expected of the proposed enzymic subunit of hexosaminidase. Its molecular weight is a fraction of that of the complete enzyme, and it is serologically cross-reactive with antibodies to the complete enzyme. As the material cross-reacts with antiserum precipitating both hexosaminidase A and hexosaminidase B we assume that it has a structure closely related to common features of those two antigens. It can be predicted that the specific antihexosaminidase A serum described by Beutler could be used in a similar fashion to locate the specifier subunit characteristic of that enzyme if this theory is correct.

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Intrinsic Semiconduction, Electronic Conduction of Polymers and Blood Compatibility

Considerable efforts are being made to understand better the interaction of blood with synthetic as well as natural surfaces and to develop materials that are compatible with blood[1–5]. The compatibility of blood with surfaces has been associated chiefly with ionic charges based on the observations that the endothelial wall, the platelets and the plasma proteins carry net anionic charges in normal physiological conditions[6]. The possible role of electronic conduction and semiconduction in blood compatibility, by contrast with mere ionic interactions, has not, however, been demonstrated before. I describe here observations in support of such a role.

In 1946, Szent-Györgyi[7,8] suggested that proteins may have an electronic structure similar to that of semiconductors. Evans and Gergely[9] calculated that this is possible although they presented no conclusive evidence. In 1953, Eley *et al.*[10] reported semiconductivity in albumin, fibrinogen, and edestin.

Synthetic polymers are typically insulators rather than conductors of electricity. Some polymers can be made "conductive" by the dispersion of finely powdered metal particles or carbons, or by coating them with metal oxides. Polyurethanes filled with carbon have been reported which showed higher thromboresistance than those without carbon[11]. Although these and other polymers containing dispersed conducting particles are sometimes inaccurately referred to as "conducting" plastics, their conductivity is attributable to the presence of the dispersed extraneous particles within the polymer matrices rather than to the intrinsic structure of the macromolecules.

I have previously reported in detail the thermal conversion of a specially purified aromatic polyimide, poly[N,N′-(*p,p*′-oxydiphenylene)pyromellitimide], into semiconducting and conducting condensation products[12–16]. In addition to intrinsic electronic conduction and semiconduction, the brittle materials retain their original shapes after weight loss during pyrolysis, have smooth surfaces, contain no leachable impurities, and in the powdered state show unusual adsorptive properties in aqueous organic solutions[17].

The thermal conversion process is accompanied by the appearance of an electron paramagnetic absorption (EPR) spectrum caused by the formation of free radicals[13]. The calculated free spin concentrations (g^{-1}) are greater than 10^{19}, and the relative EPR adsorption curves exhibit characteristic maxima as functions of time and temperature of the pyrolysis[12–14]. During the thermal conversion the resistivity of the system decreases (conductivity increases) and the density increases[12–14]. The thermal conversion process is accompanied by cleavage of the carbonyl groups of the polyimide and formation of condensed polynuclear aromatic and heterocyclic systems with enhanced π-orbital overlap and consequent increase in the mobility of electrons[12–14].

In subsequent work, the Lee-White blood coagulation times, partial thromboplastin times (PTT), and platelet aggregations and activation were determined with human blood. The two former blood values were compared with those of siliconized glass tubes[18]. The partial thromboplastin times are indicative of the activation of blood coagulation factors. The data in Table 1 indicate that the semiconducting and conducting polymers have clotting times two to three times longer than that of siliconized glass, whereas the nonconducting original polyimide has a clotting time of approximately a third that of siliconized glass. The partial thromboplastin indices show similar trends. Using plasma rich in platelets[19], the electroconducting and semiconducting polymers show very low or no platelet aggregation with no evidence of activation when compared with the original, insulating polyimide. No *in vivo* tests with animals have been carried out but they are planned in collaboration with others.

Although it is premature to generalize, if subsequent chemical, physical, and biological experiments further confirm the results, they will open up new horizons in the understanding of the interaction of blood with synthetic surfaces, leading to practical applications. They may also have implications as far as arteriosclerosis and the molecular properties of the vascular endothelium are concerned. It is possible that electroconduction and semiconduction are involved in the interaction of surfaces with plasma proteins, and activation of the Hageman factor and the platelets by an unknown mechanism.

Table 1 Physical and *in vitro* Biological Properties of Insulating, Semiconducting and Electroconducting Polypyromellitimides (PPMI)*

Sample	Density ($g\ cm^{-3}$) (refs. 12, 13)	Specific electroconductivity† $(ohm\ cm)^{-1}$, 25° C (refs. 12, 13)	No. of spins g^{-1} (refs. 12, 13)	Lee-White coagulation index‡	PTT index‡	Platelet aggregation and activation
Unmodified PPMI	1.426	$<10^{-12}$	—	0.3	0.5	Heavy and activated
Pyrolitic PPMI	1.33	0.1	$>10^{19}$	2.1	1.3	Very light to none, not activated
Pyrolitic PPMI	1.48	11.1	$>10^{19}$	2.6	1.3	Very light to none, not activated
Pyrolitic PPMI	1.65	20.0	$>10^{19}$	2.5	1.4	Very light to none, not activated

* Poly(N,N′-(*p,p*′-oxydiphenylene)pyromellitimide].
† Specific electroconductivity = 1/specific resistivity.
‡ Expressed as ratio of clotting times for test specimens and those of siliconized glass (average of three).

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Intestinal Adenomatosis in the Pig: Occurrence of a Bacterium in Affected Cells

ENTEROPATHY of the weaned piglet, originally described by Biester and Schwarte[1], has been further reported by Rowland and Rowntree[2]. Affected pigs show adenomatous proliferations of the intestinal mucosa, principally of the lower ileum and caecum. The affected tissue consists of glands lined by vigorously proliferating immature epithelial cells throughout the entire depth of the mucosa, with loss of villi and often the development of frankly polypoid masses. Goblet cells do not form. In the absence of secondary infection, most animals recover within about 6 weeks and the intestine is normal at slaughter. Histochemical studies have shown the adenomatous cells to be lacking the more common enzymic activities of normal mature villous epithelium.

Immunofluorescent studies, using a fluorescein isothiocyanate (FITC) conjugated serum prepared from an affected pig, appropriately absorbed with pig tissue, have demonstrated, in the cases so far examined, particulate fluorescence in the apical cytoplasm of affected cells throughout the depth of the mucosa. These animals originated from several farms.

Further proof of the specificity of this reaction is afforded by the removal of fluorescent activity from the serum after absorption with affected intestinal mucosa but not normal intestinal mucosa from the same animal.

Electron microscopic examination of conventionally prepared and cryostat material has consistently identified an irregularly curved bacterium within abnormal cells situated in the same areas in which fluorescence has been observed. Attempts to identify the presence of these organisms by the usual bacterial stains, either in section or in smears of affected intestinal mucosa, have been unsuccessful. The ultrastructure of the intracellular bacterium is simple and we have been unable to observe any specialized cell structures, such as axial fibres, which have been present in intracellularly located spirochaetes in swine[3].

Cultural examination of affected intestinal mucosa has resulted in the isolation of an organism with a similar morphology to that seen by the electron microscope in the adenomatous lesions. These bacteria have the characteristics of a vibrio, but initial studies suggest that the organisms differ biochemically from most vibrio species isolated from the pig by other workers[4-7]. The vibrios obtained from adenomatous lesions show a degree of antigenic homogeneity, although preliminary work indicates some antigenic differences between isolates. In contrast, none of these organisms is agglutinated significantly by a series of five antisera (unpublished results of G. H. K. L. and J. Hannah) prepared against whole cells of isolates considered to be *V. coli*, recovered from the large intestine of pigs. These latter have characteristics similar to those described by Lussier[7]. The vibrio occurring in the proliferative intestinal lesions has been demonstrated to be present in three cases, from which it has been isolated in numbers approximating to 15×10^6 per g weight wet tissue.

Hyperimmune serum against the vibrio isolates has demonstrated, using a "sandwich" technique, specific intracellular fluorescence at a similar site to that observed using conjugated porcine immune serum.

Within the cell, the organisms are not contained within vacuoles or other membrane-bound structures. This relationship seems unusual, although bacteria have recently been described free in the cytoplasm of affected hepatic cells of the Mongolian gerbil in Tyzzer's disease[8].

One must be extremely cautious at this stage in drawing conclusions regarding any interaction between the intracellular bacteria and the proliferative intestinal changes observed, especially in view of the long and controversial history of the possible pathogenicity of various vibrio species in the pig. Even in the absence of specific cause and effect, however, the host-parasite relation in these circumstances may prove of considerable interest and possible significance.

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Pepsin Inhibitory Activity amongst Activation Peptides of Pepsinogen

THE concept of the pepsin inhibitor (PI) and a method for its isolation in crystalline form from the acid-activated pepsinogen (Pg) system, in which pepsin (P) and other peptides (pp) are released (Pg→P+PI+pp), were advanced by Herriott[1]. PI was believed to interact with pepsin at pH 5 to 6, this inhibitory interaction being most readily observable in this pH range in the milk clotting system in which pepsin is believed to catalyse the reaction κ-casein→para κ-casein+caseinomacropeptide.

PI was found[2] to be a basic peptide of twenty-nine amino acid residues with leucine in the N-terminal position, but the amino acid composition originally reported does not now correspond to any single stretch of pepsinogen sequence as recently determined[3].

We report here on the peptides separated by a chromatographic method after release from porcine pepsinogen activated at pH 2 for 1 min. One of the peptides (AP-V, Fig. 1), has pepsin inhibitory activity at pH 5.7 in the milk clotting system equivalent to the inhibitory activity of PI, but differs from PI in amino acid structure and in having fewer amino acid residues, although both have the same N-terminal amino acid, leucine.

Porcine pepsinogen, prepared according to Ryle[4], was activated at pH 2; the released pepsin was inactivated and removed by filtration after precipitation by trichloroacetic acid and the residual activation mixture adjusted to pH 6 and chromatographed on CM-'Sephadex'. Salt (sodium acetate) gradient elution yielded fractions, each of which was examined for pepsin inhibitory activity in the milk clotting system[1], for ninhydrin reactivity[5] and for absorbance at 280 nm. Fig. 1*a*, *b* shows the chromatographic separation which was obtained; chromatograms are separated for clarity.

The AP-V peptide fraction was isolated by freeze-drying after desalting on 'Sephadex' G-25 (using 2.5% acetic acid), and an amino acid analysis was carried out.

Inspection of Fig. 1*a*, *b* shows clearly that AP-V, having marked ninhydrin and milk clotting inhibitory activity, is of immediate significance and it was found to contain arginine (2), aspartic acid (1), glutamic acid (1), leucine (4), lysine (4), proline (1), serine (1), valine (3). Judging also by the absence of isoleucine, glycine, threonine, histidine, phenylalanine and tyrosine, it is equivalent to the first sixteen amino terminal residues of porcine pepsinogen reported[3] to contain the highly basic -Arg-Lys-Lys- sequence.

Figure 1*a* shows that ninhydrin reactivity of the fractions is not necessarily accompanied by pepsin inhibitory activity and *vice versa*.

In spite of the sharing between AP-V and the crystalline PI of a common N-terminal leucine and equivalent pepsin inhibitory activity, the variety of amino acids and the number (twenty-nine) of amino acid residues reported by Van Vunakis and Herriott[2] together preclude common identity for these two inhibitors. Also, work[3] on the amino acid sequence of pepsinogen reveals less correspondence between PI and pepsinogen than between AP-V and pepsinogen.

The distribution of pepsin inhibitory activity amongst activation peptides of pepsinogen is the subject of continuing investigation in this laboratory but it seems that very intense inhibitory activity, for example AP–XI (Fig. 1*a*, *b*), can be associated with what, on the basis of ninhydrin reactivity, might be concluded to be a minor peptide.

In another experiment using SE-'Sephadex' (after Rajagopalan *et al.*[6]) it has been possible to elute (0.1 M NH_4OH) basic peptides which are devoid of pepsin inhibitory activity. In similar work, but using 'Amberlite' IR-120, such peptides were found[3] to contain at least one lysine or arginine residue, and even in one peptide the very basic -His-Lys-His- sequence. Neither of these two groups reported on pepsin

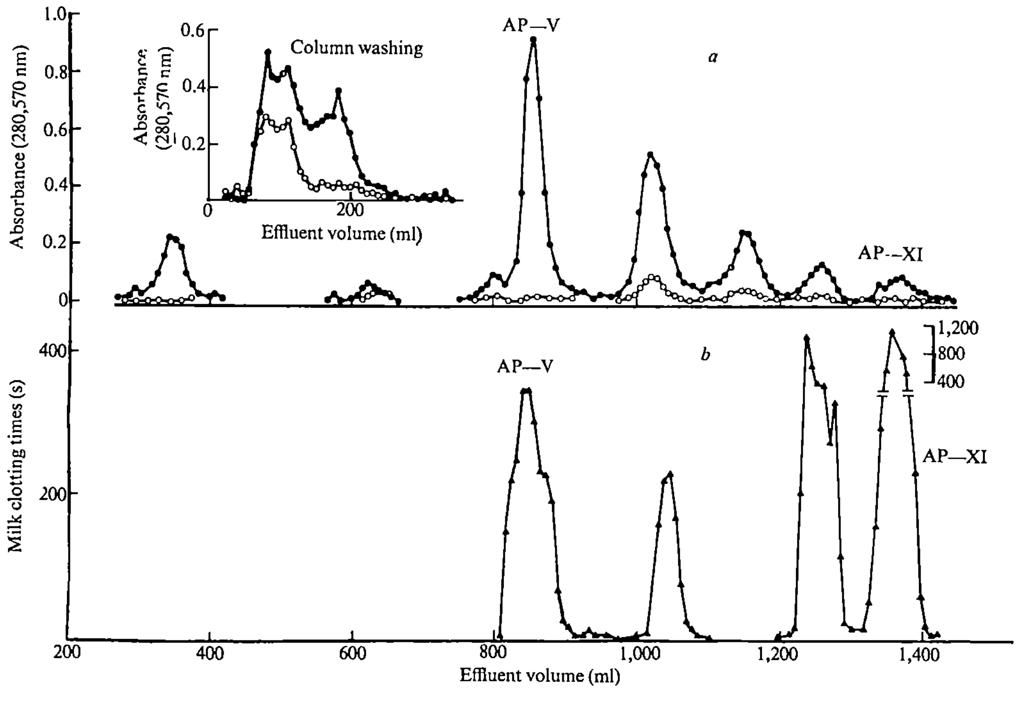

Fig. 1 Chromatography at 4° C of the activation peptides from 500 mg of chromatographically purified pepsinogen on a column (2.54 × 40 cm) of CM-'Sephadex' G-25 (Pharmacia Ltd, London; particle size 40–120 µm, capacity 4.5±0.5 meq g^{-1}) equilibrated with 0.1 M sodium acetate buffer (pH 6.0). The peptides were produced by activation of pepsinogen (500 mg 35 ml^{-1} water; 5 N hydrochloric acid to pH 2.0, 1 min). Liberated pepsin was removed by filtration after denaturation (5 N sodium hydroxide to pH 11 to 12, 5 min) and precipitation (3 N trichloroacetic acid to pH 2.0, 20 min). The resulting solution (approximately 80 ml) so obtained was adjusted to pH 6.0 and applied to the column, which was then washed with 200–300 ml of the equilibrating buffer. Elution was carried out with an exponential acetate concentration gradient formed by passing 1 M sodium acetate buffer, pH 6.0, into a constant volume 1 l mixing vessel containing 1 l of 0.1 M sodium acetate, pH 6.0. Rate of flow was 30 ml h^{-1} and 2 ml fractions were collected. Pepsin inhibitory activity of 0.25 ml of the fractions was assayed by the milk clotting method of Herriott[1] modified by the use of a pepsin concentration of 0.2 mg ml^{-1} for convenience; milk clotting times (test-control) are a direct measure of pepsin inhibitory activity. Ninhydrin reactivity[5] (0.5 ml aliquots) and absorbance at 280 nm of the same fractions were measured. ▲—▲, Milk clotting times (s); ●—●, ninhydrin reactivity (absorbance at 570 nm); ○—○, absorbance at 280 nm.

inhibitory activity for these basic peptides. Assuming that the non-inhibitory peptides separated in this laboratory are analogous, and owe their basic character, to the amino acids noted by Perlmann[3], the absence of pepsin inhibitory activity which we find supports the contention that inhibitory interaction with pepsin at pH 5 to 6 requires in the peptide something more than the presence of basic amino acid residues, a fact that has been foreshadowed[7] in pepsin inhibitory studies of lysine, which is inactive, and polylysines, in which inhibitory activity can, to some extent, be related to molecular weight[8–10].

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Specific Neutralization of Human Hepatitis Type A in Marmoset Monkeys

THE transmission of human hepatitis type A (infectious hepatitis) to marmoset monkeys, previously reported from this laboratory[1–5], has been confirmed by several other laboratories[6–9] but questioned by one[10,11]. To strengthen the case for considering the agents causing experimental hepatitis in marmosets as viruses of human hepatitis type A, and not as latent "marmoset hepatitis viruses" as has been suggested by the dissenting laboratory[10,11], it was necessary both to show clearly the lack of induction of disease by normal human serum or plasma and to demonstrate specific neutralization by convalescent human serum of the hepatitis type A agents transmitted from man to marmosets. We describe such neutralization here.

After the original transmission of hepatitis type A to marmosets, several experiments to test the reliability of the system were performed with coded specimens. In these experiments human serum or plasma obtained during the acute phase of hepatitis regularly caused hepatitis in marmosets whereas control or pre-infection serum or plasma did not (Table 1). Other experiments established some of the physical and biochemical characteristics of one of the hepatitis virus isolates[5]. These characteristics (with the possible exception of heat stability) were similar to those established epidemiologically and by human volunteer studies of human hepatitis type A viruses[12]. Similar results have been obtained recently with hepatitis type A viruses isolated in another laboratory[13]. Cross challenge of marmosets with various hepatitis isolates showed that there were at least two different antigenic types of hepatitis A viruses[14] (Table 2). Yet these data were not sufficient to establish the identity of the agents isolated in marmosets as human hepatitis A viruses.

Table 1 Inoculation of Coded Human Specimens into Marmosets

No. of experiments	Species of marmoset	Type of inoculum	Number of specimens	Marmosets hepatitis/ inoculated
5	*S. fuscicollis*, *S. nigricollis*	N	8	0/52
		H	11	47/56
1	*S.* (*Oedipomidas*) *oedipus*	N	2	0/7
		H	2	5/9
Totals 6		N	10	0/59
		H	13	52/65

N, Normal human serum albumin or control human serum or plasma; H, acute phase hepatitis A serum or plasma.

Table 2 Cross-challenge of Marmosets with Various Hepatitis Isolates*

First inoculation type	Second inoculation type	Result
GB	GB	No disease
GB	Berlin	No disease
GB	MS-1	Hepatitis
Berlin	GB	No disease
WW-55	GB	No disease
MS-1	MS-1	No disease
MS-1	GB	Hepatitis

* For origin of hepatitis isolates see refs. 1, 3 and 7. All animals developed biochemical and morphological evidence of hepatitis after the first inoculation. They were reinoculated 2–12 months after their serum enzyme activities had returned to normal. Each animal was challenged with 10^3–10^4 infectious doses intravenously and was evaluated as previously described[1].

Attempts were therefore made to neutralize the disease in marmosets with convalescent human serum[15]. Acute phase serum or plasma from human volunteers inoculated with the MS-1 strain of human hepatitis A (ref. 16), or the infectious fractions of such serum or plasma prepared by density gradient centrifugation in caesium chloride[5], were mixed in a 1:1 ratio with pre-inoculation or convalescent serum from one of the volunteers. The mixtures were incubated for 16–18 h at 4° C, and were then inoculated into groups of marmosets (*Saguinus nigricollis, S. fuscicollis*);

Table 3 Neutralization of Hepatitis A in Marmosets

Inoculum	Marmosets hepatitis/ inoculated	Average incubation period (days)
M_A+control	9/12	45
M_A+M_C	1/8	110
F+control	4/6	26
F+M_P	4/4	28
F+M_C	0/6	—
K_A+control	3/5	42
K_A+M_C	0/6	—

Control, human serum albumin; M_P, preinfection plasma from volunteer M; M_A, acute phase plasma from volunteer M; M_C, convalescent plasma (90 days postinoculation) from volunteer M; K_A, acute phase plasma from volunteer K; F, infectious plasma fraction. Three aliquots (5 ml. each) of a pool of acute phase plasma from three volunteers (M, K and R) who had been inoculated with the MS-1 strain of human hepatitis A were centrifuged at 30,000 r.p.m. for 3 h at 20° C in a SW-50 rotor of a model L Beckman ultracentrifuge. Each pellet was resuspended in 5.0 ml. of CsCl (1.1958 g ml.$^{-1}$) and was recentrifuged in the same rotor at 30,000 r.p.m. for 24 h at 20° C. Fractions were collected and fractions of the density range of 1.18–1.22 g ml.$^{-1}$ were pooled and bovine serum albumin was added to a final concentration of about 1%. The pool was then dialysed for 12 h against distilled water and for an additional 6 h against phosphate buffered saline at a pH of 7.2. The pool was mixed with the various materials as indicated in the Table and was incubated and inoculated as described in the text.

0.5 ml. of the mixture was administered intravenously to each animal. These experiments were performed under code, the code being kept by one of us (M. C.) until all results were recorded. The results are given in Table 3. Neither human serum albumin nor pre-inoculation serum inhibited the infectivity of the acute phase serum or its infectious fraction, whereas the convalescent serum neutralized the infectivity of these inocula almost completely.

Similar observations have been made simultaneously and independently in Dr Hilleman's laboratory with hepatitis isolates obtained from cases of epidemiologically proven hepatitis A occurring in Costa Rican children. These isolates were neutralized by convalescent but not pre-inoculation sera from the same group of volunteers (who had been inoculated with MS-1 hepatitis) used in our study, and they were also neutralized by the convalescent serums of contact cases from the Costa Rican study but not by pre-illness sera of the same individuals[13].

The results of both investigations, and of previous studies, establish that human hepatitis type A viruses can be transmitted to some species of marmosets, inducing biochemically and morphologically typical hepatitis. This finding eliminates the need for human volunteers in most further studies of hepatitis A, and should lead to complete identification of and preventive measures against hepatitis type A viruses.

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Relation between Apatite Solubility and Anti-cariogenic Effect of Fluoride

THE free enthalpy of formation of solid solutions of hydroxyapatite (OHA) and fluorapatite (FA) at 25° C

$$Ca_5(PO_4)_3OH_{1-x}F_x \tag{1}$$

has been determined[1]. I have used these thermodynamical data to calculate (Driessens, unpublished) the solubility of the solid solutions in aqueous solutions at certain *p*H values. For this purpose, the following relations were derived:

$$Ca_5(PO_4)_3OH_{(s)} \rightleftarrows 5\ Ca^{2+}{}_{(l)} + 3\ PO_4^{3-}{}_{(l)} + OH^-{}_{(l)} \tag{2}$$

$$-\log K^1_{OHA} = 5\,p\text{Ca} + 3\,p\text{PO}_4 + p\text{OH} = -\log K_{OHA} - \log a_{OHA} \tag{3}$$

$$Ca_5(PO_4)_3\ F_{(s)} \rightleftarrows 5\ Ca^{2+}{}_{(l)} + 3\ PO_4^{3-}{}_{(l)} + F^-{}_{(l)} \tag{4}$$

$$-\log K^1_{FA} = 5\,p\text{Ca} + 3\,p\text{PO}_4 + p\text{F} = -\log K_{FA} - \log a_{FA} \tag{5}$$

where subscript (s) means that the preceding molecular unit is part of the solid solution; subscript (l) means that the preceding ion is dissolved in the aqueous solution; K^1_{OHA} and K^1_{FA} are the apparent solubility products of the components OHA and FA over the solid solution respectively: they depend on x; K_{OHA} and K_{FA} are the solubility products of pure OHA and pure FA respectively: they are independent of x; a_{OHA} and a_{FA} are the thermodynamical activities of FA and OHA, which are sufficient to account for the dependence on x.

Data for the activities were derived by application of the appropriate graphical method to the literature data of the free enthalpy[1]. Then the solubility (s gmol of apatite per litre) was calculated at *p*H 4, 6 and 8 as a function of x on the assumption

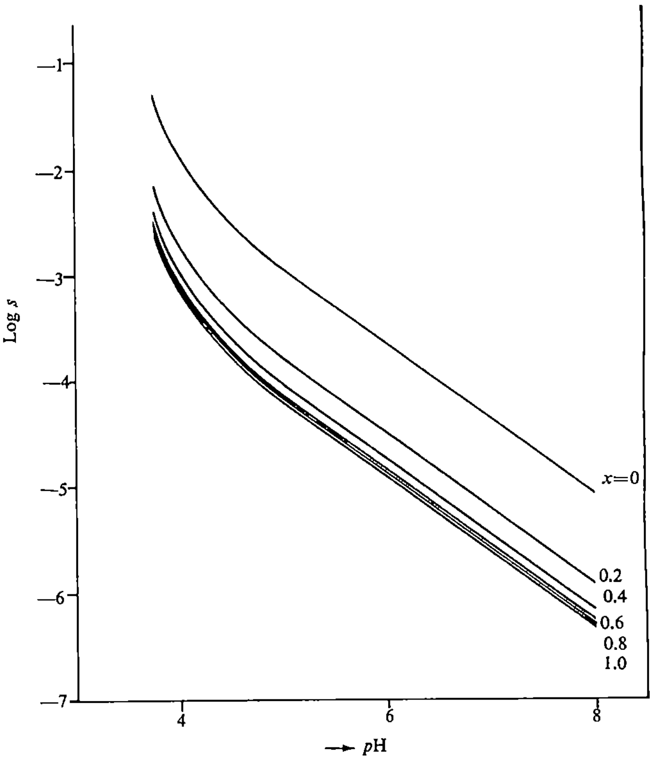

Fig. 1 Solubility s in gmol of apatite ($Ca_5(PO_4)_3OH_{1-x}F_x$) dissolved per litre at 25° C as a function of *p*H for a number of compositions x of the OHA-FA solid solutions at equilibrium.

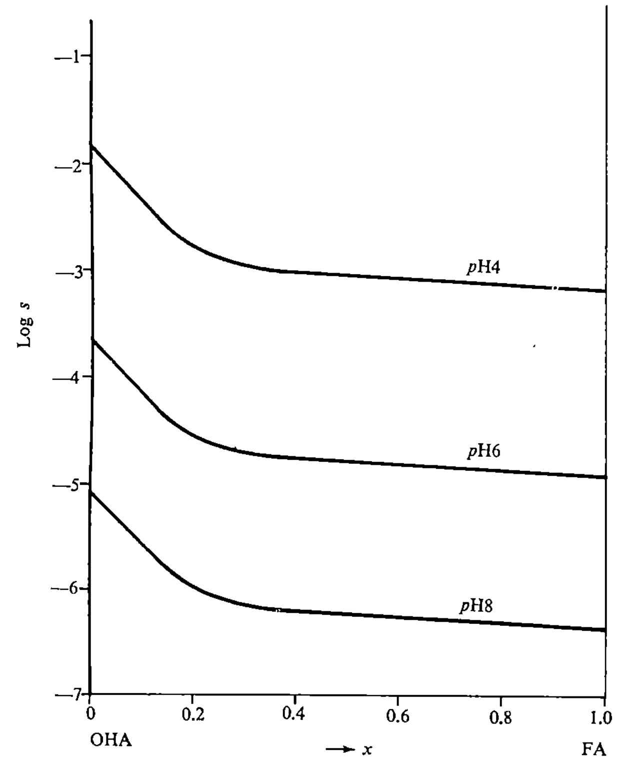

Fig. 2 Solubility s in gmol of apatite dissolved per litre at 25° C as a function of the composition x of the OHA-FA solid solution at pH 4, 6 and 8.

that the aqueous solution would not contain Ca^{2+} or phosphate ions other than from the original OHA-FA solid solution. If necessary, corrections were made for the occurrence of binuclear complexes, such as $CaHPO_4^0$, $CaH_2PO_4^+$, in the aqueous solution. The results are given in Figs 1 and 2. Values for K_{OHA} and K_{FA} were taken from Moreno, Gregory and Brown[2] and McCann[3]. Values for the stability constants of binuclear complexes were taken from Gregory, Moreno and Brown[4].

The calculated data show that the reduction of the solubility of hydroxyapatite at a certain pH in the range $4 \leq p\text{H} \leq 8$ by the incorporation of F^- ions is nearly independent of pH but depends strongly on x. The reduction of the solubility as compared to that of pure hydroxyapatite amounts to 44, 68, 80 and 87% at the following degrees of substitution of OH^- by F^- ions: $x = 0.05$, 0.10, 0.15 and 0.20 respectively.

Although the apatite in tooth enamel is not a pure OHA-FA solid solution, it may be expected that the incorporation of small amounts of fluoride ions has at least the same effect on its solubility. In fact, detectable preventive action has been observed when drinking water contained 1 p.p.m. F^-. On the other hand, such a concentration and dosage lead to a value of about $x = 0.05$ within a short period after eruption of the teeth[6]. Therefore, the reduction of the apatite solubility by F^- incorporation seems to be a sound basis to explain the anticariogenic effect of fluorides.

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Bacterial Production of Di-Methyl Nitrosamine in Salted Fish

The presence of the potent experimental carcinogens, N-nitrosamines, in food and their possible formation during food processing have recently received much attention. N-nitrosamine formation from the interaction of nitrite with secondary amines in mammalian stomach[1,2] and *in vitro* by the action of nitrate-reducing bacteria has been reported[3,4]. This *in vitro* effect was confined to bacteria of the intestinal flora. Reports concerning N-nitrozation of secondary amines by bacteria isolated from food are scarce. Collins-Thompson *et al.*[5] demonstrated the production of N-nitrosamines *in vitro* by bacteria isolated from meat products.

We have reported the presence of di-methyl nitrosamine (DMN) and di-ethyl nitrosamine (DEN) in appreciable quantities in Chinese salted marine fish obtained from markets in Hong Kong[6]. This was attributed to bacterial action on nitrate in the crude salt used to prepare salted fish. We have obtained viable culture on salt agar of nitrate-reducing *Staphylococcus aureus* and halobacteria.

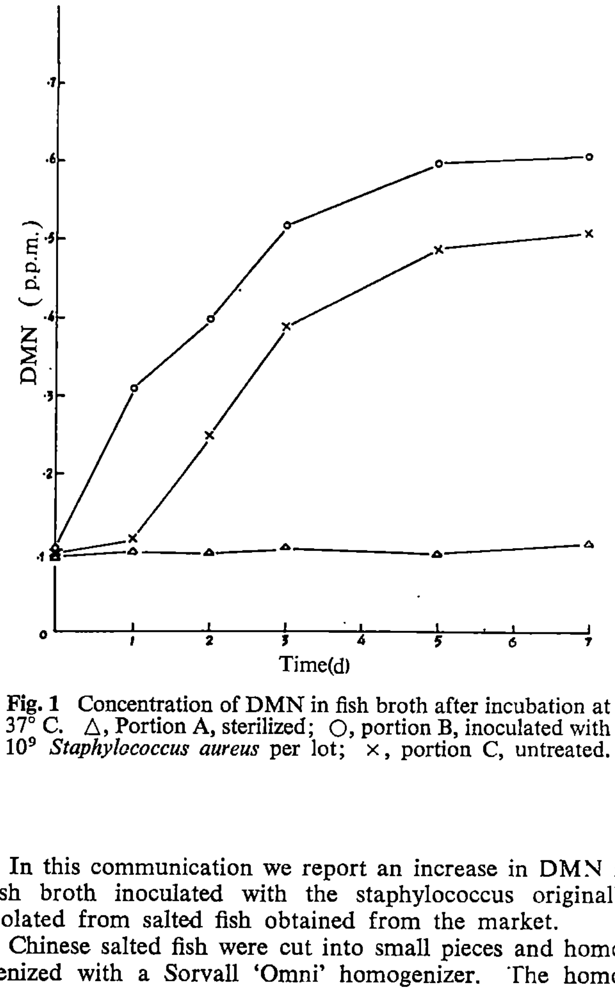

Fig. 1 Concentration of DMN in fish broth after incubation at 37° C. △, Portion A, sterilized; ○, portion B, inoculated with 10^9 *Staphylococcus aureus* per lot; ×, portion C, untreated.

In this communication we report an increase in DMN in fish broth inoculated with the staphylococcus originally isolated from salted fish obtained from the market.

Chinese salted fish were cut into small pieces and homogenized with a Sorvall 'Omni' homogenizer. The homogenate was divided into three equal portions—A, B, and C. Each was subdivided into six equal lots in conical flasks. They were treated as follows. Portion A was sterilized by heating in a water bath at 70° C for 1 h daily. Portion B was sterilized by the above method on 0 day and 10^9 *Staphylococcus aureus* in 1 ml. were added to each lot. Portion C was untreated.

The nitrate content in the fish broth was 40 p.p.m. and nitrite content 5 p.p.m., estimated according to the method of Follet and Ratcliff[7].

The material was incubated at 37° C. One lot from each portion was taken out and analysed for DMN content on day 0, 1, 2, 3, 5 and 7. The method of extraction of DMN was described by Sen *et al.*[8]. For identification, a Varian aerograph model 2868 was used, and identification was done on two different columns: (*a*) 5% SE on 80–100 mesh high performance grade 'Chromosorb W (AW-DMCS)' $\frac{1}{8}$ inch × 6 foot stainless steel column. (*b*) 10% 'Carbowax 20 M' on 80–100 mesh high performance grade 'Chromosorb W (AW-DMCS)' $\frac{1}{8}$ inch × 10 foot stainless steel column.

The extract was further examined and the identity of DMN confirmed by the combined GC-MS technique. The concentration of DMN was calculated from the gas chromatogram peak area and the intensity of the molecular ion of the mass spectrum. The results are shown in Fig. 1. A shows no change in the DMN content of the fish broth; B and C show progressive increases, with the curve flattening towards the end of the 7-d period. The rise in the DMN content of portion C lags 1 d behind that of B, but thereafter C parallels B.

Our study shows conclusively the important role of the staphylococcus originally isolated from fish in the production of DMN from its precursors in the fish. The amount of DMN present would thus seem to be dependent on the storage condition, degree of contamination by nitrate-reducing bacteria, and the amount of precursors present. These features are in keeping with our observations (Fong and Chan, unpublished results) that different batches of fish from the market show a considerable variation in N-nitrosamine content. The study described here, however, does not resolve the question whether the part played by the bacteria is enzyme-dependent[4] or non-enzymatic as reported by Collins-Thompson *et al.*[5].

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Wear of English Monumental Brasses caused by Brass Rubbing

THERE are about 8,000 monumental brasses in the churches of Britain, representing only a small proportion of the total number of brasses set in place between about 1250 and 1650 (ref. 1). The preparation of rubbings of many of these brasses is extensively practised at present, particularly by visitors to England. Although brass rubbing (in the Netherlands) is documented as far back as 1656, the use of a wax crayon on paper has been popular in Britain only since the early nineteenth century[2]. Thus, when viewed in historical perspective, the increasingly popular brass rubbing activity must be considered as a comparatively new feature in the history of the monumental brasses. Our study was undertaken to determine whether repeated brass rubbing over periods of the order of a century will result in significant brass wear caused by the friction of the back of brass rubbing paper against the brass.

Neutron activation analysis is a sensitive means of measuring exceedingly small amounts of copper transferred from the surface of the brass to the back of the brass rubbing paper as a result of the microscopic motion of the paper against the brass during rubbing. The amount of transfer per unit area can be considered as a measure of the wear on the brass caused by making a rubbing.

Before beginning our experiments in Britain, we made several trial rubbing experiments at NBS on cleaned, freshly polished and freshly machined brass surfaces. On both kinds of brass surfaces measurable quantities of copper were transferred to the back of the paper. We report here a series of controlled experiments carried out on representative monumental brasses in Norfolk, England.

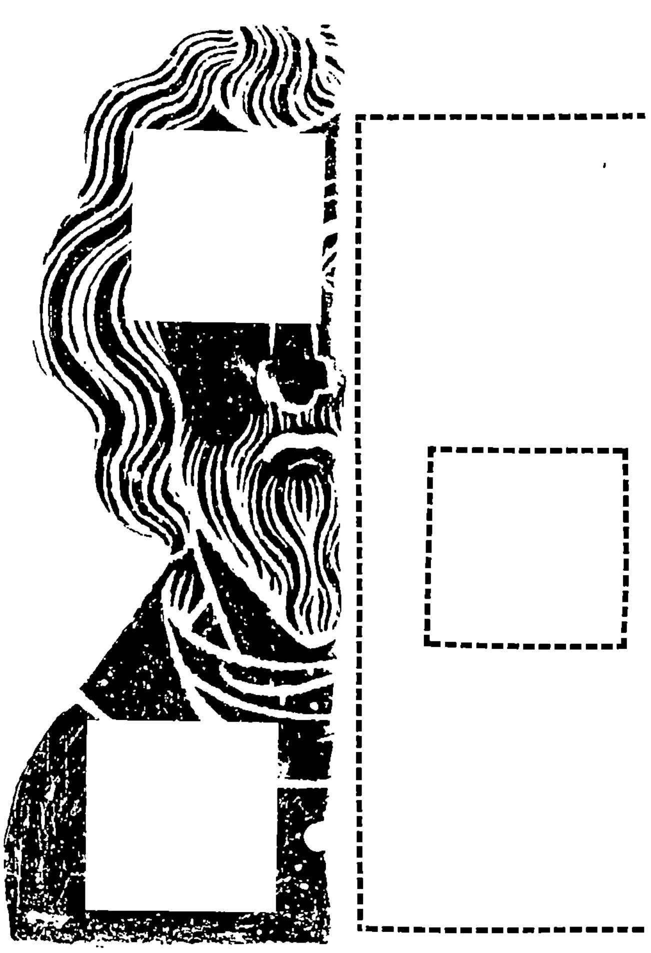

Fig. 1 Typical rubbing and associated control following sampling for neutron activation analysis. Two samples have been cut from the rubbed area and one from the control area. Head is of James de Hoveston, Blickling Church, Blickling, Norfolk (*c*. 1360).

Multiple rubbings from ten brasses in four churches in Norfolk were made using white paper selected for its low content of Cu. We have shown in separate control neutron activation experiments that the black crayon used (Astral heelball) did not contribute significant amounts of Cu to the paper. Before making a rubbing, the brass was lightly brushed with a clean brush and a piece of 8.5×11 inch paper, selected from the centre of the ream, was laid on the brass. One portion of the paper was constantly protected from the brass by interposing a second sheet of the same Cu-free paper; this protected section was used as an analytical control in each case. Fig. 1 shows a typical rubbing, and associated control, after removal of samples for neutron activation analysis. The brass was rubbed with heavy pressure until further rubbing caused no visible changes in the intensity of the rubbed regions. Each finished rubbing and its associated control were stored in separate sealed polyethylene bags without folding.

The sealed packets were opened at the National Bureau of Standards in a lamillar flow dust-free hood. Two or more square (25 cm^2) samples were cut from each sheet. Each sample was folded and rolled up, then hermetically sealed in a medical grade polyethylene tube. Seventy-two samples (rubbings plus controls) were prepared. Two standard solutions of copper were prepared by dissolving weighed quantities of high purity Cu metal in dilute HNO$_3$, and these standard solutions as well as the samples were loaded into polyethylene "rabbits". Neutron activation inducing the reaction ^{63}Cu(n,γ) ^{64}Cu was accomplished by irradiation for 60–120 s in RT-4 of the NBS 10 MW reactor (neutron flux=1.2×10^{13} cm^{-2} s^{-1}). After irradiation the samples were stored for >16 h to permit the decay of active short half-life radiation. Each sample was removed, folded flat, and sealed between two sheets of non-irradiated polyethylene, providing reproducible counting geometry. Known volumes of the standard irradiated copper solution were used to saturate folded filter paper which was counted in the same configuration.

The radiation counter was a Li-drifted Ge detector (2.4 keV resolution at 1.332 MeV). The amount of activity caused by decay of ^{64}Cu (half-life=12.8 h; detection of the 0.511 MeV γ-ray due to positron annihilation) was monitored for each sample during counting times of 2–5 min. The number of counts was corrected for a 0.511 MeV positron annihilation γ-ray arising from Na impurity in the sample. This correction was 2.5% of the number of counts in the 1.36 MeV Na peak, and ranged from 2–20% of the number of ^{64}Cu counts, depending on the copper content of the samples. The total number of ^{64}Cu counts in a 5 min interval was approximately 400 for a blank and up to 7,700 for a rubbed sample with a high content of Cu. All data were corrected for the decay of radioactivity in the time interval between irradiation and counting.

Table 1 shows the basic data obtained in this experiment. The fraction of the sample area rubbed in direct contact with the brass surface is determined by the extent of detail incised in the region of the brass selected for study; this parameter was measured after counting using the dark areas on the rubbing samples, and varied from 1.00 for smooth areas to 0.26 for highly decorative regions. The measured copper content has been tabulated on a normalized basis by dividing the total copper content found by the fractional area rubbed.

A total of twenty-seven control samples were analysed for Cu in this study, and these values were found to be remarkably uniform (0.039 (±0.007) μg Cu cm^{-2}). In each of the forty-five rubbing samples studied the copper content was larger than that found in the associated blank.

After counting, the reverse side of each sample was carefully examined visually and, in certain cases, small flecks of gold or silver-coloured material were detected. Chemical tests have established that these metallic-coloured flecks are most likely due to tiny particles from crayons used in previous brass rubbing activities. Separate neutron activation measurements have shown that by contrast with black crayons, metallic-coloured crayons contain appreciable quantities of copper. Counting excised flecks from some contaminated samples, we found that much of the total Cu content was contained in the flecks. (Samples in which such contamination is suspected are designated by an asterisk in Table 1.)

In Fig. 2 we show the frequency distribution of the data on Norfolk brasses. Data are separated into two sets, primary and secondary, based respectively on the visual absence or presence of metallic-coloured flecks. The primary set of rubbing samples has a median Cu content near 0.12 μg Cu cm^{-2} and a range from 0.018–0.39 μg Cu cm^{-2}. The secondary set has a median Cu content near 0.25 μg Cu cm^{-2} and exhibits a broader distribution.

If we adopt the median value of about 0.12 μg Cu cm^{-2} as typical of wear arising from brass rubbing, we can convert this number to a typical thickness of brass removed per rubbing. English monumental brasses of the period studied contain about 65–80% copper by weight[3]. Assuming a brass density of about 8.5 g cm^{-3}, we find that 0.12 μg Cu cm^{-2} corresponds to a thickness of about 1.8×10^{-8} cm of brass removed per rubbing, assuming uniform loss. If we assume that a popular brass will be rubbed three times per day on average, then in one century $\sim10^5$ rubbings will be made, and the wear associated with this number of rubbings will amount to $\sim1.8\times10^{-3}$ cm. This seems a trivial amount of wear by comparison with the usual depth of incision on many brasses ($\sim$0.1 cm), although the later (eighteenth century) copper plates sometimes have very shallow engraving[4]. Our primary set of data indicates a maximum Cu transfer about three times as great as the typical value chosen above. Even this maximum observed value of Cu transfer corresponds to a trivial amount of brass degradation over a century of brass rubbing at a rate of three rubbings per day.

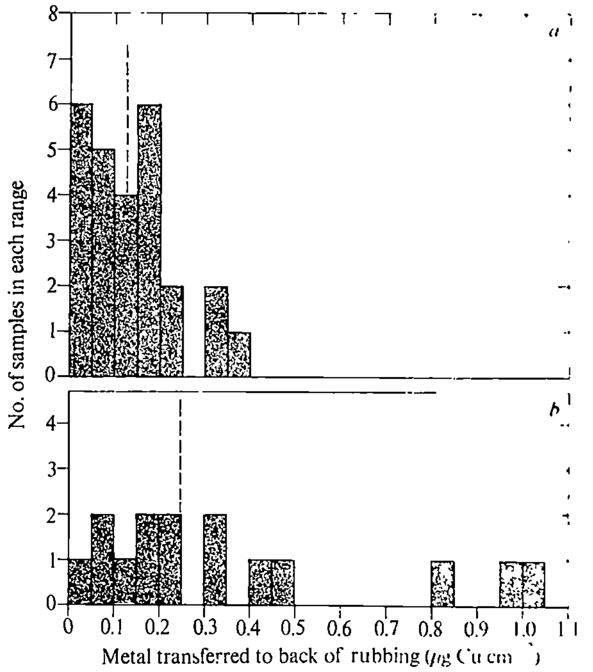

Fig. 2 Frequency distribution of copper transfer from English monumental brasses. - - -, Median Cu transfer. *a*, Primary set of samples; *b*, secondary set, involving possible extraneous Cu contamination.

There is a point of caution to be emphasized in the interpretation of these data, however. We have measured only one aspect of brass degradation caused by brass rubbing

Table 1 Measurement of Copper Transfer to Back of Brass Rubbing Paper for Representative Brasses in Norfolk

Date of brass manufacture	Brass identification, location and description	Fraction of sample area rubbed	Cu (μg cm^{-2}) (above control)
c. 1360	James de Hoveston, Blickling Church, Blickling		
	Head	0.80	0.035
	Head	0.90	0.018
1401	Sir Nicholas Dagworth, Blickling Church, Blickling		
	Stomach	0.96	0.49*
	Stomach	0.99	0.34*
	Stomach	0.97	0.197*
	Mail	0.47	0.127
	Mail	0.66	0.059
	Sword belt	0.74	0.087*
1416	Sir Simon Felbrigg, Church of St Margaret, Felbrigg		
	Face	0.95	0.154
	Banner	0.26	0.31
1432	Robert Baxter, Mayor, St Giles Church, Norwich		
	Hand	0.83	0.154
	Robe	0.80	0.20
	Robe, repeat	0.84	0.110
	Foot	0.82	0.317
	Foot	0.86	0.206
	Inscription	0.79	0.151
1432	Christiana Baxter, St Giles Church, Norwich		
	Head	0.91	0.176
	Hands	0.86	0.167
1458	Cecilie Boleyn, Blickling Church, Blickling		
	Skirt	0.80	0.97*
	Skirt	0.68	0.83*
	Skirt	0.65	0.322*
	Skirt	0.65	0.14
	Inscription	0.70	0.41*
	Inscription	0.70	0.39
1485	Isabel Boleyn Blickling Church, Blickling		
	Skirt	0.75	0.224
	Skirt	0.62	0.156*
	Inscription	0.61	0.125
c. 1500	? Robert Gardiner, St Andrews Church, Norwich		
	Upper robe	0.84	0.043
	Upper robe	0.64	0.025
	Lower robe	0.97	0.041*
	Centre robe	0.81	1.05*
	Centre robe	0.81	0.054
	Hands	0.84	0.047
c. 1500	? Wife of Robert Gardiner, St Andrews Church, Norwich		
	Middle robe	0.71	0.073
	Lower robe	0.72	0.100
	Lower border	0.90	0.049
1612	Thomas Windham, Church of St Margaret, Felbrigg		
	Detail near hand	0.72	0.246*
	Detail near hand	0.73	0.228*
	Detail near hand	0.62	0.115*
	Hand	0.81	0.075
	Central region	0.72	0.072*
c. 1960	NBS sample, washed with ethanol		
	Smooth face	1.00	0.045
	Smooth face	1.00	0.019
	Rough face	1.00	0.066
	Rough face	1.00	0.051

* Possible contamination by metal flecks from brass rubbing crayons left from previous brass rubbings.

and the general access of brasses to the public. Other types of degradation must surely exist, such as wear by rugs which are trodden on and moved repeatedly, the effect of brass flexure (with possible associated work-hardening and eventual fracture (H. K. Cameron, personal communication)), and corrosion caused by skin acids. Our results must not therefore be interpreted as indicating that monumental brass degradation from all possible causes added together is necessarily trivial.

We thank Dr H. K. Cameron, Mr John Smith, Mr John Page-Phillips, Rev. J. R. Southern, Rev. and Mrs Hedleigh Davis and Mr Tom Gills for their help in our study.

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Impurities in Saccharin and Bladder Cancer

We reported[1] that a combination of dietary saccharin with a single dose of methylnitrosourea (MNU) produced bladder tumours in five out of twelve female Wistar rats. No tumours were observed in control animals receiving either saccharin or MNU alone. Since submitting this report, concern has been expressed at the United States Food and Drugs Administration meeting, May 17, that impurities in saccharin, particularly *o*-toluene sulphonamide, could be responsible for the bladder tumours observed in saccharin feeding studies at the Wisconsin Alumni Research Foundation (WARF)[2,3].

Saccharin manufactured by the same process as that used in the WARF studies, namely by the Remson-Fahlberg process, in which *o*-toluene sulphonamide is the starting material, is known to contain this compound as an impurity. A very recent analysis of the saccharin used in our experiment showed it contained 810 p.p.m. of *o*-toluene sulphonamide. No evidence is at present available that this level of *o*-toluene sulphonamide is significant, but the possibility that it is this, or some other impurity, rather than saccharin *per se* which acts as the co-carcinogen in our experiments, will be further investigated. The saccharin used in our work is representative of that currently sold not only in the UK, but also in many other parts of the world[3].

We thank Dr A. W. Noltes, Coca Cola Europe, London, for arranging the analysis done by the Battelle Institute, Columbus, Ohio, USA.

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BOOK REVIEWS

Predicting the Weather

Weather Forecasting as a Problem in Physics. By Andrei S. Monin. Translated by Paul Superak. Pp. x+199. (MIT: Cambridge, Massachusetts and London, December 1972.) $12.50.

MOST readers of *Nature* will be aware that weather forecasting has recently been undergoing very substantial changes in technique. The older empirical and partly subjective methods (not lacking physics but unable to exploit it numerically to any great extent) have become largely, though by no means wholly, replaceable by quantitative methods deriving from direct application of the basic laws of physics. The problem posed is to observe the 3–D atmosphere, or some part of it, in regard to weather-significant properties like pressure, temperature, humidity at a given initial instant and then to calculate its evolution by using dynamical and thermodynamical equations and boundary conditions (a model) thought to represent, more or less adequately for the particular kind of forecast required, the physics of the evolutionary process. (For experimental purposes initial data may also be invented.) Because of the non-linearity of the equations the numerical solutions are only to be obtained by finite-difference methods on high speed, large capacity computers, so that massive computational as well as physical problems are involved.

The intensive development of the new techniques has been an important concern of atmospheric dynamical research since the Second World War and has involved academic as much as or more than weather-service scientists. (This research is blessedly almost free from categorization as "pure" or "applied".) But the only textual literature in English specifically on the subject has been that provided back in 1961 by P. D. Thompson's *Numerical Weather Analysis and Prediction* (Macmillan) so that great interest should be aroused by the appearance now of a second volume and that from a Soviet scientist who is very distinguished for his research on turbulence theory but had not perhaps been thought of in the West as greatly concerned with large-scale atmospheric dynamics. Dr Monin shows here that he is as familiar with our literature in this field as with his own to which he has indeed contributed significantly.

Monin's book is in four sections. A short introduction deals with the history of the subject and with the scales and spectra of atmospheric processes. This is followed by a chapter on the theory of short-range weather prediction which in physical terms is to use an adiabatic model and in practical terms a model useful for two or three days since the run-down time of the atmosphere is a week or so. The use of quasi-geostrophic, quasi-solenoidal approximations which filter out the "non weather producing" motions from the system is fully discussed and advocated against the current tendency to use the more complete ("primitive") equations.

The author then passes to problems of long-range prediction, global climate and climatic change, for which non-adiabatic processes—radiation, friction and turbulent fluxes—are very much at the heart of the matter. These latter, strangely enough for Monin, are not given much attention except for radiative heating and cooling. There are discussions of a global system of observation such as will be used for the Global Atmospheric Research Programme, of predictability, and of possible mechanisms for climatic change. A final section deals with laboratory modelling using differentially heated rotating liquids, with the atmospheres of the planets out to Jupiter and with the utilization of forecasts.

This is a scholarly work in which few words are wasted. There is indeed such compression, in parts describing the construction of models, that some familiarity with the field by the reader is desirable but at least he does not have to face large amounts of intricate mathematics. The writing is engagingly fresh with the author ready to express controversial views both about the science and its uses which will raise some eyebrows. Yet it also has a somewhat pedagogic quality with here and there a lack of balance, as if the writer were passing on received knowledge rather than the results of work with which he has been much concerned. The practising forecaster might thus find the presentation occasionally irksome and he would mainly seek in vain for examples of the applications of the techniques described, except in long term, global models. But the scope of the treatment is admirable and will most certainly be enjoyed by all with some knowledge of the subject. The book should also be an excellent means of informing physicists who would like to be made aware without needless complication of the way in which the subject is heading.

The translation is reasonably idiomatic but one remains pleasantly aware that the work was originally written in Russian.

P. A. SHEPPARD

Visual Physiology

Physiology of Photoreceptor Organs. Edited by M. G. F. Fuortes. Pp. x+765. (Springer: Berlin and New York, 1972.) 244 DM ; $77.40.

"VISUAL science has undergone remarkable development in the past two decades. A generation of competent, vigorous workers, skilled in the use of the latest biophysical and biochemical techniques, has built a structure of new understanding on older foundations that puts this branch of science in the very forefront of neurophysiology." Thus aptly, H. K. Hartline, who so very appropriately contributes the introduction as well as an article with F. Ratliff, summarizes the significance of this new volume of the *Handbook of Sensory Physiology*. What more can a reviewer reasonably say in a short space. to show that all the essays are welcome and many of them thoughtful summaries of a busy subject? Fuortes has picked his topics and contributors well. The literature up to about 1970 is discussed and, of course, one can hear the voices and read the reviewers commenting on how dated some of the articles are already. That may be so for the specialist reading the essay of his specialty. But I do not find this especially disturbing. The requirements of a handbook such as this are that the bibliographies shall be accurate and extensive and the articles readable. Thus it is possible to find out what is going on in specialities adjacent to one's own.

For example essays such as that by A. Kropf on the structure and reactions of visual pigments and W. R. Levick on the receptive fields of retinal ganglion cells are welcome not so much to the specialist but for their readability by those primarily interested in the structure of the compound eye of insects (O. Trujillo-Cenoz) or retinal metabolism (W. Sickel).

In all there are 18 chapters and this volume can fairly share the shelves with its great German progenitors. But whose shelves? It is understandable that publishers are not philanthropists and that book production of this standard is expensive. Yet it is hard to comprehend the economics of publishing such a generally useful book at over £30 a copy. If the answer be that libraries will buy it, the reply will soon be not all that many, at this price in the present waning economic circumstances of libraries. Perhaps *Nature's* readership could come up with some ideas for publishers for different costing, before more of us find the only way we can economically get a useful new book on our scholarly shelves is to review it briefly.

B. B. BOYCOTT

Neural Biochemistry

Neurotransmitters and Metabolic Regulation. Organized and edited by R. M. S. Smellie. Pp. ix+169. (The Biochemical Society: London, 1972.) £4; $12.

THE organizers of the symposium upon which the present volume is based, and the editor, should be congratulated for having produced a well balanced and up to date collection of reviews on biochemical aspects of neurotransmission. The eight chapters cover all of the known neurotransmitter substances, and at the same time pinpoint many of the topics of greatest current research interest in the field as a whole. Thoenen gives an admirable review of the new area concerning the neurally mediated control of enzyme synthesis in adrenergic neurones. He discusses the effects of trans-synaptic influences and the protein nerve growth factor in controlling the activities of the biosynthetic enzymes tyrosine hydroxylase and dopamine-β-hydroxylase in adrenergic neurones. Watkins describes, mainly from a biochemical viewpoint, the metabolism of the amino-acid transmitters, glycine, gamma-aminobutyrate, aspartate and glutamate, and discusses the biochemical complexities resulting from the transmitter and metabolic functions of these substances, and how these functions may be related. McIlwain, and Greengard and McAfee, deal with the roles of adenine nucleotides, such as adenine, adenosine and cyclic AMP, in mediating the actions of neurotransmitters and in controlling neuronal excitability, a complex and fast-changing literature with a paucity of good reviews of this type. Whittaker and his colleagues, and Smith, discuss recent knowledge of the biochemical mechanisms involved in the uptake, storage and release of acetylcholine (in electric fish and in squid) and of noradrenaline in sympathetic nerves. For these systems, and for other transmitters, remarkable similarities in the basic design of the biochemical mechanisms are emerging. In cholinergic and adrenergic nerves special uptake systems exist for choline and for noradrenaline respectively; the intraneuronal storage vesicles in both types of nerve contain not only high concentrations of the transmitter substances but also soluble proteins (chromogranins and vesiculin) so far with ill-defined but probable functions in transmitter storage. Banks and Mayor review an equally topical field, the axonal transport of macromolecules and cell organelles, illustrating by reference to their studies in adrenergic neurones. Carlsson and his colleagues review the regulation of the synthesis and degradation of 5-hydroxytryptamine by nerve activity in tryptaminergic neurones in mammalian central nervous system, and the various control mechanisms that may exist in such neurones.

Altogether the volume comprises a timely and useful collection of topical reviews of key areas, at a modest price. The volume is clearly illustrated and well produced, and should provide much valuable information to those involved in research or study in this rapidly growing field.

L. L. IVERSEN

Bacteriocins A-Z

The Bacteriocins. By P. Reeves. Pp. xi+242. (Chapman and Hall: London; Springer: Berlin and New York, March 1973.) £5.80.

BACTERIOCINS, the antibacterial agents produced by bacteria, have been of continuing interest to bacteriologists and biochemists since their discovery in 1925. Many early investigations concerned the identification of new types and their medical implications, such as the role of bacteriocins in recovery from infection and in control of normal flora. Currently, the mode of killing by colicins, the agents formed by *E. coli*, is of particular interest at the biochemical level, as is the phenomenon whereby a bacteriocinogenic bacterium is partly immune to its own bacteriocin. In many species, the genetic determinants of bacteriocin synthesis have turned out to be carried on autonomous extrachromosomal genetic elements—plasmids—many of which are related to other bacterial plasmids like the F factor or the drug-resistance factors of enterobacteria. Plasmid molecules have revealed new structures for replicating DNA and new forms of control of DNA replication.

This monograph has seven chapters dealing with bacteriocins in general and with colicins in particular; the chemistry of bacteriocins; a shortish passage on the structure and behaviour of the genetic determinants; and much fuller sections on the adsorption of bacteriocins to bacteria and how they cause death. An appendix lists the known bacteriocins down to rarities like the "convexins" of *Eggerthella convexa.* The twenty-one-page bibliography includes titles as well as the last page number of each paper. Monographs can be all-embracing or written to a theme. This one is undoubtedly of the first sort, and will be very useful to everyone working in the field.

G. G. MEYNELL

Physics of the Sea

Marine Physics. By R. E. Craig. Pp. vii+83. (Academic: New York and London, February 1973.) £1.90.

THE difficulty in reviewing this little book is to know on what level to assess it. One of its aims, as given by the author, is to provide some background on physical aspects of the sea for engineers and biologists. He anticipates criticism of the unevenness of its treatment by stating that the different chapters do not depend greatly on each other: each is an essay on its own topic.

The term "density currents", in the first chapter, is taken to include large-scale geostrophic currents maintained by density differences in the ocean but the brief treatment, even when supplemented by later chapters, fails to indicate the importance of such flows. The most detailed chapter is that on wind currents in deep water, which gives a quantitative account of simple types of motion in the presence of Coriolis forces, leading on to atmospheric and oceanic flows involving viscous stresses. This is the classical Ekman spiral treatment with some original touches. It is taken for granted in this chapter that the reader is familiar with differential equations, but that on waves starts by assuming a cycloidal surface wave form in deep water with closed circular orbits and the phase velocity and attenuation with depth are derived on this basis. The equations of motion and continuity are not mentioned and there is no reference to the existence of a mass transport. The chapter on tides avoids almost entirely any quantitative treatment and gives a rather superficial descriptive account.

The last two chapters, on optics and acoustics, are elementary but soundly based and best meet the description of being brief, self-contained essays on their respective topics. The lack of consistency of treatment would make this book of limited value as a textbook, but taking it as a collection of essays on topics in marine physics the discriminating reader may find some items of interest.

K. F. BOWDEN

NATURE Volume 243
JUNE 22 1973

Scientific Freedom and Hunger Strikes

SUNDAY, June 10, 1973, marks a sad day indeed in the history of the Soviet Union. On that day seven scientists, all Soviet citizens, started a hunger strike designed to bring attention to the fate of scientists who, in the recent past, have attempted fruitlessly to obtain permission to emigrate to Israel. Whether this situation is any worse now than it was a few years ago cannot really be gauged but the fact that the Soviet Union is now relatively open to communication by telephone makes it easier for the rest of the world to hear of, and appreciate, the conditions in which the Soviet scientific profession works.

One of the latest sufferers of injustice is Professor Mark Azbel who was, until recently, head of the Department of Electron Theory of Metals at the Landau Institute of Theoretical Physics in Moscow. Professor Azbel's treatment at the hands of Soviet officialdom is so similar to treatment meted out in the past to other members of the Soviet scientific community that it is only the name that needs to be changed in accounts which *Nature* has published more than once in recent years. What is sad in the case of Professor Azbel, however, is the fact that he and his wife have been separated from their 12 year old son, a point brought out most poignantly in a letter from him dated June 8, addressed to "Scientists of the World":

"For 20 years I was engaged in science in the Soviet Union. I want to live the rest of my life in Israel. My 12 year old son is already there. He got his permission on March 27, and yet I am not allowed to leave the USSR. I am not accused, as many others are, of being involved in the secret activities, possibly because of the topics of about 100 of my papers which were published in the scientific magazines all over the world and which were submitted to these magazines from the well-known institute. I happen to be guilty just in being the doctor of science who must not be useful anywhere outside the USSR, and I am condemned to be separated from my son and to be forcibly detained in the USSR.

"I do not know who passed this sentence on me. I do not know for how long it will act. I cannot even appeal against it. I have not got any written sentence. It was told to me only orally and only by the irresponsible visa official, the only secret court of our time who announces the most essential judgement in the life of the Jew who wants to live in Israel—without any law, any lawyers, any defenders, any publicity and even without any written sentence. Will the world accept such a bible of inquisition customs, such absence of any control, such arbitrariness? Are the scientists and their societies so weak as to allow the scientific degree to become the stamp of state property? Will the lawyers and their international organizations be silent? I cannot be silent. I appeal! Scientists over the world help me! I can never get rid of my doctor's thesis. Does it mean I shall never see my son in Israel?"

Not only are the Soviet authorities apparently determined to bring to a halt the career of any scientist who deigns to apply for permission to emigrate to Israel, but it seems that an element of the treatment is to disrupt families and to bring personal unhappiness into their lives. It is only two weeks since *Nature* (**243**, 313 ; 1973) highlighted the unfortunate case of Dr Evgeny Levich who was whisked away from his family and, as far as can be ascertained, is still at Tiksi in the Arctic, carrying out a period of military service. Now Professor Mark Azbel and his wife are faced with the agonizing decision of whether to leave their son in Israel or to attempt to reunite the family in the Soviet Union which, in all probability, would put an end to all further thoughts of emigration.

But what do the seven Soviet scientists hope to achieve with their hunger strike? By their own words they intend to prove that they belong to nobody but themselves. They state that the Soviet Union claims them as its own property and so "As a matter of principle and provided all other ways are exhausted, [we aim] to destroy this property rather than recognize that anybody is entitled to our souls and bodies". They will end their hunger strike, according to reports, if they are granted exit visas. But it must be asked if this hope is not fulfilled whether in fact there are no other ways of helping these men. The scientific community would suffer a grievous loss if they became martyrs for this cause. This is not to say that the importance of their campaign should be underestimated, but brave men who are prepared to die for their beliefs will do their cause more good if they stay alive to fight.

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100 Years Ago

AS a supplement to the extract from James Forbes' "Orienta Memoirs," given by Dr. Gulliver in NATURE (vol. viii. page 103), the following incident, recorded by Capt. Johnson, deserves republication :—

" I was one of a party at Jeekarry, in the Bahar district ; our tents were pitched in a large mango garden, and our horses were picqueted in the same garden at a little distance off. When we were at dinner, a Syce came to us complaining that some of the horses had broken loose in consequence of being frightened by monkeys (*i.e. Macacus Rhesus*) on the trees. . . As soon as dinner was over, I went out with my gun to drive them off, and I fired with small shot at one of them, which instantly ran down to the lowest branch of the tree, as if he were going to fly at me, stopped suddenly, and coolly put his paw to the part wounded, covered with blood, and held it out for me to see. I was so much hurt at the time that it has left an impression never to be effaced, and I have never since fired a gun at any of the tribe.

G. J. R.

from *Nature* **8**, 163; June 26, 1873

NASA's Shuttle Shuffle Endangers Space Science

EVEN before its first flight, the space shuttle has demonstrated a remarkable capacity for dodging hostile fire. Some 18 months ago, in response to bitter opposition from the Office of Management and Budget over the costs of the shuttle, NASA officials radically altered its design, choosing a model which will be only partially recoverable in place of the fully manned, reusable launch vehicle that was originally on the drawing board. The switch cut the estimated development costs in half, secured support from OMB and easily won approval from Congress in a key vote which allowed NASA to let contracts for the project. Undaunted, however, the shuttle's critics in the Senate have continued to attack NASA's economic analysis of shuttle operations in the hope that they can shoot enough holes in it to persuade Congress to delete funding for the project when NASA's budget comes up for approval. But they have found that one of their key weapons—a critical report prepared by the General Accounting Office—has been badly blunted by yet another abrupt change of tack by NASA (see *Nature*, **243**, 372; 1973).

In short, NASA was claiming last year that use of the shuttle will save some $5,200 million between now and 1990 when compared with the costs of a comparable space programme using conventional, expendable launchers. But the GAO was about to publish a report criticizing NASA's figures and suggesting that cost overruns and other uncertainties may reduce the claimed economic advantages of the shuttle, when NASA came up with a completely fresh analysis of shuttle operations which, it is claimed, will save as much as $16,000 million. The agency has yet to make a formal announcement of its new mission model, and it has yet to provide details of how those remarkable savings are to be made, but according to one NASA official, the chief change from previous plans is the idea of using the shuttle for sortie missions with the spacelab which may be developed by ESRO. The spacelab will cut the cost of payload development and thereby allow NASA to make more shuttle flights. In that case, it is not difficult to see why NASA is anxious that ESRO should develop the spacelab.

But, as important as costs are to members of Congress, this complex debate over constantly shifting economic analyses to some extent misses the most important question about the shuttle: is it the best vehicle for carrying out a productive and useful space programme? So far, NASA's promotion of the space shuttle has been a clear case of putting the cart before the horse. Instead of first choosing what objectives should be accomplished in space in the 1980s, and then planning a launcher system to fit in with those objectives, NASA has sold Congress the concept of the shuttle and is now discussing what it will be used for. It has, for example, asked the Space Science Board of the National Academy of Sciences to offer advice on how the shuttle can best be used for scientific missions. It goes without saying that such a study should have been made two years ago, and it is not sufficient to argue that political realities forced NASA first to sell the concept, and then come up with the justification.

There have been signs, however, that critics of the shuttle in the Senate have changed their line of attack, and are asking some embarrassing questions about the effect of the shuttle on other space activities in the next few years. Their questions deserve a better answer than NASA has so far given. In short, they are concerned that the projected costs of developing the shuttle will squeeze out more valuable and productive programmes.

That concern has gained considerable momentum from a table supplied by NASA to Senator James Abourezk which outlines the runout costs of all the programmes contained in NASA's budget request for 1974 (in other words, the table does not include any estimates for new projects). The table shows costs of the shuttle increasing from $200 million last year, to $1,100 million in 1976 and $1,190 million in 1977, while the run-out costs of space science and applications programmes decline steadily from $868 million last year to $422 million in 1977.

The worrying thing about the table is that although it takes no account of any possible new programmes, it shows no decline in NASA's total spending until after 1977. The clear implication is that unless NASA is able to persuade the Office of Management and Budget and Congress of the need for a larger total budget, there will be no money for new projects until after spending on the shuttle peaks in 1977. The consequences of such a hiatus are surely sufficiently alarming to require an explanation from NASA.

But the standard response from NASA officials is that they are sure that the agency's total budget will soon get back to the level of two years ago—about $3,400 million. But the cold fact is that this year the Office of Management and Budget trimmed NASA's budget request to $3,100 million. Spending projections for next year suggest a total budget of $3,160 million and there is absolutely no guarantee that it will pick up again.

What then would be the consequences if NASA's optimism turns out to be unjustified, and there is no money for new programmes for the next few years? The High Energy Astronomy Observatory has already been suspended, and its missions considerably reduced in scope. The Grand Tour of the Outer Planets has gone by the board, as have several of the Apollo missions that were expected to be most productive and two Orbiting Solar Observatories have been scrapped. Those projects were dropped partly because of budgetary pressures in the past few years, but also because NASA would not be able to support them and the shuttle without a large budget increase. In a sense, therefore, the shuttle has already cut deeply into the space science programme, and it will cut even more deeply if there is no money available for the Venus Pioneer mission, which will be up for funding next year, or even for the revamped HEAO missions. NASA's nimble footwork in dodging criticisms of the shuttle has thus secured approval for the project, but it has also seriously endangered the health of space science for the next few years.

OLD WORLD

Trouble still Brewing over Law of the Sea

British proposals affecting the law of the sea have been heavily criticized by the Parliamentary Group for World Government. The all party group, which embraces both houses of Parliament, declared last week that the proposals would "effectively remove from mankind the richest part of its heritage, endanger freedom of scientific research and increase the likelihood of international conflict".

The proposals that have aroused the group's fury provide for a grid system—of the type used to regulate oil prospecting in the North Sea and elsewhere—which will divide up the area of the world's ocean into administrative areas for exploitation.

The proposal has been about for some time, but the group's action appears to be prompted by the start, on July 2, of the final preparatory meeting for the United Nations' Law of the Sea Conference planned for Santiago next year.

Under the British proposal—which is by no means guaranteed approval by the Santiago conference—the world's oceans would be divided up into large rectangles which any nation could license from the International Sea Bed Authority that is to be set up by the Santiago conference. Each nation could then exploit its part of the oceans. The proposal also provides that only a small percentage of the oceans would be licensed initially—perhaps five or ten per cent—in order that the developed nations do not gain further economic advantages over the under developed.

To further ensure what is described as an "equitable distribution", the licences would be granted on a quota basis, which takes into account population.

Developing countries would also be able to sub-licence companies in developed countries to actually exploit sea bed resources for them, benefiting both from the immediate return on the work and from the experience of the technology.

The parliamentary group ridicules this scheme describing it as a twentieth century enclosure act and predicting that it will cause international conflict. Unfortunately the group has nothing to put in place of the British proposal.

There is, however, an alternative, and currently it seems to have more explicit support than the British proposal. Its advocates include many of the developing countries and its aim is to create an International Sea Bed Authority that will actually exploit the oceans itself, distributing the profits after administration costs.

Objections to the scheme—largely voiced by the developed countries—are that the cost of setting up such a commercial organization to exploit the minerals and hydrocarbons beneath the seafloor would be astronomical. Sums larger than the entire budget of the United Nations would be needed, Britain has claimed.

Defining the constitution of such an authority might well prove an insuperable task, as it would have not only to to be strong commercially, but would have to be able to resist political pressure—for example from copper producing countries who might try and restrict the mining of manganese nodules. Equally, past experience with international organizations does not suggest that a commercially orientated one would be any more successful than its diplomatic predecessors.

The parliamentary group also warns of the dangers presented to scientific research by other proposals before the conference. Extension of "patrimonial seas" to 200 miles from land—a proposal that may well be approved in one form or another at Santiago—could hamper scientific research, the group claims, and in doing so it is reflecting well-founded fears that the scientific community has been expressing for some time (see *Nature*, **239**, 421 ; 1972). A large block of the developing countries, notably the South Americans and Africans, are claiming that scientific research is merely a mask for commercial exploitation of the seabed (in the words of a Peruvian delegate "knowledge is power and must therefore be controlled"). What the developing countries are proposing is that scientific research should be carefully regulated by the International Seabed Authority, which would not only grant permission for scientific research on the high seas, but which would also have access to all results before they were published.

Currently this proposal is being strongly opposed by the developed countries—USSR included. Firm declarations have been made that scientific research must be kept free. It remains to be seen how much the attitude of the Africans and South Americans is simply a negotiating position, but there remains a danger that scientific research could suffer if the interests of defence and economics alone rule in Santiago next year.

NATURAL RESERVES

Uranium Found in Britain

Several thousand tons of uranium oxide ore have been found in Britain. The discovery of the ore was in fact made some years ago at the start of the Institute of Geological Science's five year search for uranium which it has been conducting on behalf of the United Kingdom Atomic Energy Authority. The £0.25 million programme revealed deposits of the ore in northern Scotland, Cornwall and Devon, but full announcement of the discovery was not made until last week. A preliminary announcement that some ore was present near Ousdale burn in Caithness was made in 1970[1].

IGS is unwilling as yet to say how much ore has been found, or to say precisely where it is, although, apparently, it is not located in national parks. Dr S. H. U. Bowie, chief geochemist at IGS, said this week that while the ore is not of grade one standard, it still represents an "important strategic reserve".

The current price of recovered uranium oxide is just under $10 a pound. The price will have to rise to between $10 and $15 a pound before it becomes economic to mine the newly discovered deposits. At present there is a world surplus of uranium but by the mid-1980s it is expected that the price —which has remained stable for some time—will begin to rise. At that point the British deposits might prove worth mining.

Although they are small in comparison with the estimated world deposits (100 million million metric tons, 2.5 million million of which are within a mile of the surface), they might, if they proved to be in the order of 8,000 tons, provide enough energy to meet the equivalent of the whole of the United Kingdom's current electricity demand for about 50 years. Whether in fact the find will be good for that long will not be known until the IGS provides more details, probably later this year.

The five year programme, which ended in March, involved searching five areas of Britain—northern Scotland, the southern uplands of Scotland, the Midlands, Central Wales and south western England. Before the search began the likeliest sites were considered to be Devon and Cornwall and northern Scotland, including the Caithness and Orkney areas.

[1] Institution of Mining and Metallurgy Transactions B, **79**, 180 ; 1970.

RESEARCH AND DEVELOPMENT

Cambridge Science Park

CAMBRIDGE is shortly to open a science park. By the autumn the first light industry concentrating on technology advanced products that require a high degree of research and development will be installed on a site to the north of Cambridge.

The scheme, sponsored by Trinity College, emerged after a university committee under Sir Nevill Mott had recommended, in 1969, that science based industry in Cambridge should be expanded.

The report was well received, and Trinity, largely because it had some derelict land to spare, sufficient capital and an enthusiastic bursar who is also a scientist, took on the task of planning and building the park.

When it is completed the 13.5 acre site could house more than 10 companies, although initially Dr John Bradfield, Trinity's bursar, hopes to see about six companies move in. The first of these will be Laser-Scan. Set up by a group of physicists from the Cavendish laboratory, the company specializes in designing and building computer guided laser beams for counting nuclear particles. Other types of industry that Dr Bradfield hopes will be attracted to Cambridge are, for example, companies working on amino acid sequences, thin metal films and any similar science based work that requires intensive research and development.

The object of the science park is a two way exchange of ideas and personnel. Research students, the theory runs, will benefit from contact with high technology industry and the industry will benefit from having some of the best research facilities in the country at hand. Professors will be able to keep in touch by acting as consultants, and Cambridge may gain up to a thousand jobs.

The road to the park's opening has not been entirely smooth, however. The town had to be talked into accepting a development that went against its declared plans, and the college had to cope with rising building costs, but now that the first buildings are going up and service facilities are being put in, Dr Bradfield is beginning to advertise for clients confident that the scheme is more than an expensive dream.

The park hopes to attract two types of client. One will be the small, new company, specializing in a scientific field, and the other will be the highly specialized research department of large companies or public utilities. Once a company moves into anything resembling large scale production, it will have to move off the park.

The Cambridge Science Park is not unique in Britain. Other universities have close ties to science industries. But it is one of the rare deliberate attempts to create an interchange of research and production experience between universities and industry. In the United States, there are over 80 science parks, one of them more than 3,000 acres in size, but 27 of them are wholly limited to science-based industry of the type that Cambridge wants to attract. Britain offers far fewer opportuities for research ideas to be transferred directly into products.

So far, Trinity has put £200,000 into the scheme, but Dr Bradfield insists that it is not intended to be a profit making operation. "We hope it will cover its costs in the foreseeable future," he says, "but there are no plans to make a profit. It is after all an academic service."

MEDICINE

Rationalization Delayed

THE University of London's plans for the reorganization of undergraduate teaching in medicine and the biological sciences are in danger of being thwarted by the University Grants Committee. Recently the UGC turned back to the university its list of priorities in this field and the university was asked to reconsider its plans.

Following the publication of the Todd Report in 1968, which recommended that the teaching of medical students and that of biological science undergraduates should be more closely linked, the university after a long period of gestation decided on three schemes to implement these recommendations. The first plan is to place the pre-clinical and clinical medical schools of St Thomas's Hospital, the clinical medical school of Westminster Hospital and the School of Biological Sciences of King's College at one site. The second is to similarly site the pre-clinical parts of the medical schools of the London Hospital and St Bartholomew's Hospital contiguously with the biological sciences department of Queen Mary College. The third scheme is to house the pre-clinical departments of the Royal Free Hospital medical school and of the University College Hospital Medical School and the faculty of medical sciences of University College at a common site.

The University of London in its application to the UGC placed the St Thomas's, Westminster and King's scheme at the top of the list but the University Grants Committee clearly did not favour giving first priority to the most expensive scheme—which is estimated to cost about £6 milion—and the university has been asked to review its priorities. A spokesman for the St Thomas's, Westminster and King's scheme, said this week that he feared that this might mean that this scheme would now be given a low priority although he felt that there were compelling reasons why this should not be so.

One of the difficulties with this scheme has been the choice of site. After consideration of possible sites both in Covent Garden and on the South Bank, a site at St Thomas's Hospital was finally chosen but this has run into difficulties recently. Contrary to first expectations the site is not the property of St Thomas's Medical School but it is owned by the Department of Health and Social Services. Negotiations are at present taking place over what price, if any, should be paid for the site. A spokesman for the scheme said that the question of evaluating the site should not affect the plans and that it had always been understood that the site would be available.

The other schemes have not had a clear passage either and letters to the press a year or so ago made it clear that the staff at St Bartholomew's Hospital did not favour moving from their present site.

The next move is up to the University of London which has to reply to the UGC's request for a re-evaluation of the scheme.

Whether or not work starts on the proposed centre at St Thomas's Hospital on schedule depends to a great extent on the University's reply.

The present plan is that if the money is granted soon, construction can start in 1975–76 and that the first students can be admitted in the autumn of 1979.

CONSERVATION

Friends of the Whales

MONDAY sees the start of this year's meeting of the International Whaling Commission. Friends of the Earth and a whole host of other conservationist organizations are again attempting to pressure the commission into imposing a ten year moratorium. Advertisements in *The Times* next week, and letters signed by conservation organizations world wide and despatched to the heads of state of the commission's member countries are all aimed at achieving the moratorium which was approved by the Stockholm Conference 53 votes to 0 last June.

But the chances of a moratorium are no greater this year than last. Last year's meeting, small though the progress it made was, did at least move the commission some way down the road that the conservationists want it to go, and a further step this year in the form of protecting certain species further and cutting quotas again, could effectively outmanoeuvre the moratorium bid.

NEW WORLD

Double Trouble for the AEC

by our Washington Correspondent

THE Court of Appeals last week set the Atomic Energy Commission back on its heels by ordering it to produce an analysis of the environmental effects of the entire fast breeder reactor programme. It was a landmark decision in many respects, for it gives opponents of the breeder a powerful tool for opening up public debate about alternative methods of producing energy in the 1990s, and it also has important implications for other high technology ventures such at the AEC's Plowshare Programme. But that was only the first of the commission's headaches last week, for the court decision was followed swiftly by the resignation of Mr Milton Shaw, who for the past nine years has been head of the AEC's reactor development programme and one of the most powerful officials in the agency. The two events were not entirely unconnected, and it remains to be seen which will turn out to be the more important.

The court decision was the final move in a case filed against the AEC last year by the Scientists' Institute for Public Information (SIPI), a group of scientists headed by Barry Commoner and Margaret Mead. At issue is the development and possible proliferation of the liquid metal fast breeder reactor (LMFBR), the Administration's chief technological fix for the energy crisis and a prime target for criticism from environmentalist organizations.

The AEC, in conjunction with the Tennessee Valley Authority and private industry, is building a prototype LMFBR at Oak Ridge in Tennessee at a cost of some $2,000 million. The goal is to have a successful demonstration of the reactor by 1980 and to prove that it will be commercially attractive. After that demonstration, private industry will take over the reins and the AEC is projecting that by the year 2000 LMFBRs will be producing as much electricity as all the present power stations put together.

SIPI's law suit revolved around the question of whether the commission is obliged by law — specifically, the National Environmental Policy Act (NEPA)—to produce an environmental impact statement only for the pilot LMFBR, or for the commercial development that may follow. The AEC has already produced an impact statement for the pilot plant, but maintained that it would have to gaze into the crystal ball to produce an analysis of the environmental implications of the entire programme. Such an analysis, it said, "would be meaningless in terms of content".

In November last year, a lower court judge agreed with the AEC and threw the suit out, but SIPI went to the Court of Appeals where it won its case last week. The appeals court opinion, written by Judge J. Skelley Wright (who also wrote the opinion in the so-called Calvert Cliffs case which forced the AEC to consider environmental factors during the licensing of nuclear reactors), accused the commission of taking "an unnecessarily crabbed approach to NEPA". His opinion also throws up some interesting points about the development of projects involving advanced technology.

Judge Wright argues that if the AEC waits until the breeder reactor is on the verge of commercial application before it files a statement on the environmental impact of the project, so much money would be invested in the technology that

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HUMAN EXPERIMENTATION

HEW Policies Criticized

by our Washington Correspondent

THE federal government's policies for protecting the rights of those who take part in medical experiments came under fire last week in a report made public by the Department of Health, Education and Welfare (HEW). Prepared by a special ad-hoc committee set up to investigate the notorious Tuskegee Syphilis Study—a study of the effects of untreated syphilis among poor black men in Alabama—the report terms the policies vague and overly general, and suggests sweeping reforms in the system of regulating human experimentation.

Even within HEW, the committee notes that there is no uniform policy for the protection of research subjects, and points out that there is also no uniformity among other agencies of the federal government which support human experimentation. "The lack of uniformity", the committee states, "creates confusion and denies some subjects the protection they deserve".

At present, HEW relies heavily on review committees in universities and other research institutions for preserving the rights of research subjects, and the department recently issued a set of guidelines for the review committees to follow. Reliance on the committees "was an important advance", the report suggests, but the system suffers from basic defects which seriously undermine its effectiveness. In particular, the HEW guidelines are vague, have little to say about research involving children, prisoners and other groups where special considerations apply, and there are no effective procedures for enforcing them.

Accordingly, the committee suggests that Congress should establish a permanent body independent of any existing federal agency, with authority to regulate at least all federally supported research involving human subjects. The idea is that it would consist of a board, composed of people from diverse scientific disciplines and representatives from other professions, which would formulate research policies in much greater detail than is now the case, and draw up procedures to make sure that the policies are implemented by institutional review committees. The board, which the report suggests could be called the National Human Investigation Board (NHIB), would also establish appeals procedures for adjudicating disagreements between investigators and institutional review committees.

In research institutions themselves, the committee suggests that an Institutional Human Investigation Committee (IHIC) should be established as a direct link between the institution and the NHIB, and that it should have two distinct subcommittees: a protocol review group concerned with prior review of research protocols, and a subject advisory group to aid subjects with any problems concerned with their participation in the research. In particular, the subject advisory group should be concerned with procedures for making sure that a subject's consent to take part in a study is freely given after full information about the research has been provided to him.

Senator Edward Kennedy recently held a set of hearings with his Senate Health Subcommittee on the problems involved in human experimentation, during which a number of particularly glaring examples of dubious ethical practices were brought to light. He can be expected to introduce legislation soon to strengthen regulations designed to protect the rights of human subjects.

(Final Report of the Tuskegee Syphilis Study Ad-hoc Advisory Committee, available from the Center for Disease Control, Atlanta, Georgia.)

proper balancing of considerations will be difficult. In short, "technological advances are . . . capital investments and, as such, once brought to a stage of commercial feasibility, the investment in their development acts to compel their application". And, perhaps more important, by the time the breeder has reached the point of commercial feasibility, other options would be foreclosed.

Critics of the breeder reactor have long maintained that the Administration is sinking money into the project at the expense of other approaches, such as geothermal energy, solar power or controlled thermonuclear fusion.

They will thus use the court decision to stimulate a public debate about such alternative sources of energy, for NEPA specifies that environmental impact statements must consider alternative programmes.

Shortly after the court decision was handed down, Dr Dixie Lee Ray, the new AEC chairman, announced that it would not be appealed to the Supreme Court. The commission expects, however, that it will take several months to prepare the impact statement.

The court's decision has other implications outside the breeder reactor programme, and it could well prove to be one more nail in the coffin of the Plowshare Programme. Judge Wright's opinion essentially states that environmental impact statements for entire programmes must be written at the pilot plant stage. It thus seems to imply that if the AEC intends to follow up the recent Rio Blanco explosion, which was designed to test the feasibility of using nuclear explosions to extract natural gas in deep deposits in Colorado (see *Nature*, **241**, 494; 1973), it will have to outline the possible environmental consequences of complete commercial exploitation of the gas deposits. That would entail several thousand explosions, and opponents of the project are confident that when all the details are spelled out they will create devastating opposition.

As for the resignation of Mr Shaw, he is known to have been considerably upset by a recent reshuffle of the AEC bureaucracy which removed safety research and development from his jurisdiction and placed it in a division on its own (see *Nature*, **243**, 182; 1973). The decision to comply with the appeals court ruling instead of taking it to the Supreme Court was reported to be the last straw, and he decided to quit.

A powerful administrator who has incurred the wrath of the AEC's critics, Shaw has been accused in the past of paying insufficient attention to research that casts doubts on reactor safety. He has ruled the division of reactor development with an iron hand, and in so doing has won the approval of the Joint Committee on Atomic Energy because of his powerful influence in the development of nuclear power. His departure, coupled with the new arrangements for safety research, may help to assuage some of the fears of the critics of nuclear power.

In moving safety research out of Shaw's division and also in deciding not to contest the appeals court decision, Dr Ray has considerably strengthened her control over the agency. She has also established her independence from the Joint Committee—which has consistently supported development of nuclear power—and incurred the displeasure of some key members, notably Chet Hollifield, who are upset about the recent events.

NUTRITION

Protein Shortage

from a Correspondent

THE Protein Advisory Group of the United Nations System called for urgent action to meet the growing world shortage of protein at its twenty-first meeting held in New York earlier this month. The group recommended:

- The implementation of the coordinated national food reserve policies proposed by FAO.
- A global approach to world fishery management.
- Research on enhancing animal productivity and improving the yield of legumes.
- Developed countries to provide additional assistance to developing countries to help expand food production, by the provision of fertilizers, plant, equipment, etc.
- Substitution of vegetable proteins for animal proteins by, for instance, the utilization of meat-like analogues and milk substitutes.
- The development by governments of food distribution systems which ensure the supply of basic food to meet the needs of vulnerable groups.

The group's recommendations came after Mr Lester Brown of the Overseas Development Council, Washington DC, had pointed out that historically the global food situation has been largely discussed as a population and food supply problem. Now, however, rising affluence is a new claimant on world food resources. Four-fifths of the annual increase in world population occurs in poor countries, which are short of employment as well as food; in these countries the *per capita* availability of grain averages about 190 kilograms a year, most of which is consumed by people. In contrast almost one ton of grain is consumed per head of the population of the United States and Canada, but only 70 kilograms are consumed by people, the rest being used for animal production.

AIR POLLUTION

Setback for the EPA

THE Supreme Court last week ruled that much of the Environmental Protection Agency's strategy for cleaning up air pollution is illegal. The ruling, which is certain to lead to more court action, instructs the EPA to prevent state governments from allowing air quality to deteriorate significantly below present levels. It means that the agency must alter its approach to curbing air pollution.

The basis of the court case is the Clean Air Act, which lays down specific air quality standards that must be met across the United States. The EPA had approved plans drawn up by state governments for complying with the act, which allow the air to deteriorate in quality in regions where the standards are already being met, provided that it continues to stay below those standards. But the Sierra Club last year took the EPA to court, arguing that the Clean Air Act does not allow deterioration of present air quality. It won its case in a lower court in June last year, it was upheld by a court of appeals, and last week the Supreme Court was evenly divided on the issue, which has the effect of affirming the lower court ruling.

The EPA, backed by several industrial and commercial groups and some state governments, argued that unless some deterioration is allowed in air quality, there can be no industrial or population expansion in areas which have pristine air, and there also can be no relocation of industry outside the smog-ridden cities.

There is, however, some leeway in complying with the court decision, because the lower court prohibits only "significant" deterioration in air quality, without specifying exactly what significant means. The EPA is thus considering two alternative approaches. Either it will go ahead and approve state plans which would still allow deterioration, but at a level which the agency considers insignificant. That course would almost certainly lead to fresh court action in areas where environmentalists do not agree with the EPA's definition of significant. Alternatively, the agency is considering agreeing to state plans which specify that sources of pollution should use the best available technology to clean up their output of pollutants.

It seems likely that the agency will adopt the second approach, in which case it may have a difficult time arguing in court that it is preventing significant deterioration, and instead of fighting over a few areas, it will again be involved in litigation over the entire cleanup effort. It will keep the courts busy.

World Health Organization and Genetic Disorders

This critique of the World Health Organization report number 497 on *Genetic Disorders : Prevention, Treatment and Rehabilitation* is by Professor J. H. Edwards, of the Department of Human Genetics at the University of Birmingham

A COMMITTEE of cobblers, if asked to advise on the health of the world, would doubtless draw attention to the scandal of millions of naked feet and to the danger of foot-borne diseases, and advise on the need for counselling of the barefooted and for the training of both counsellors and cobblers.

This form of negative stability is by no means peculiar to health; bee-keepers, chess-players, numismatists and pot-holers share an equal missionary zeal. Even solipsists, according to Bertrand Russell, complain of their rarity.

Medical geneticists, whose report was reviewed briefly in *Nature* (**238**, 7; 1972), might be thought to be free from this hazard by their training in the biases of ascertainment, but this is not so.

The report makes a number of recommendations which are neither surprising nor inappropriate. For reasons which are not clear, however, it contains a strange mixture of doubtful assumptions, erroneous facts, and uncertain inferences, some of which overflow into recommendations. The first paragraph speaks of "the relative increase in morbidity and mortality attributable to slow changes in gene frequencies". No explanation is given. There is said to be "no reliable information on the current prevalence of genetic disease"—in fact, the information is adequate for practical purposes; the numbers of the deaf, the blind, and the ineducable are necessarily known in all countries with compulsory education, and the proportions of these due to mendelian and chromosomal disorders have been estimated on good data.

The word genic is extended from its regular use to include multifactorially determined disorders, a term used variously. In the table on multifactorial disorders, congenital malformations and conditions of unknown and "non-genetic" disease are used as exclusive categories. In Table 2 a series of malformations, mostly of unknown aetiology, are called multifactorial, which admittedly means the same thing.

Later we are redeemed with robust and orderly descriptions of biochemistry until we again leave the ground in a cloud of numerals. On page 31 a mutation rate for recessives of 10^{-4} is "assumed". On the next page $100\times5\times10^{-5}\times2+0.0001$ somehow makes 0.0086. On page 33, "genetic counselling, urging couples total abstinence from reproduction" is recommended in recessive traits, but, on page 34, we are almost all carriers—"every zygote is estimated to carry 1 or 2 deleterious recessive genes".

On page 43 the remarkable claim that fibrocystic disease can be diagnosed *in utero* is made. The claim that sickle cell disease can be diagnosed, which is also made, was hardly stronger when the committee met. On page 45 compound interest is used to provide the doubling time for "multifactorial disorders". The theory is difficult and no mention of the procedure is made, but certainly stabilizing mechanisms are likely to reduce this. The report ends on a most alarming graph, whose credentials are hardly as firm as the chronology of Archbishop Ussher, showing that the end of the world is at hand.

How, we may ask, can such an assembly of experts, with a dedicated and able secretariat, lead to the publication of a document so rich in platitude and inconsistency?

The reason is not far to seek: an assembly of experts, appropriately stratified by hemisphere, power-block and language, is expected to create, within one week, an agreed text on a subject beside which Vietnam and the Common Market are mere tea-cup storms restricted to parts of continents for a mere generation or two.

The problems presented by the genetic future of man are far more important than anything under the control of warriors, bankers, or tradesmen, and, by their nature, no executable advice can be expected from a week's discussion. What seems important is that no one should expect the committees or secretariats to undertake such impossible tasks. Given its magnitude the achievement may well be commended, but the results are not conducive to the orderly development of either practice or theory.

Perhaps the World Health Organization technical report series should restrict itself to technical reports and provide summaries of what is known, linguistic recommendations, and recipes for laboratory procedures; this may seem a rather pedestrian approach, but, in a world short of bread, elaborate schemes for cake-making should be thoroughly tested on the rich for acceptability and digestibility before being offered to the poor.

Technical reports should surely be reports of technical matters. By tackling some specific problem, such as G6PD, clotting factors, or immunoglobulins, this series has had a profound influence on the standards of human genetics throughout the world and the informal benefits of meetings of world experts have been accompanied by formal reports which have defined problems, increased the precision of words, and provided a framework from which local policy decisions could be made.

The recent report on haemoglobins shows once again the benefits which a central organization can confer simply by assembling experts and reviewing facts.

Recommendations

THE WHO Scientific Group on Genetic Disorders met in Geneva from November 16 to 22, 1971. Its chief recommendations included the following:

- medical genetics services, including counselling clinics, should be provided at medical centres;
- physicians and the public should be better educated in the principles of genetics and their relevance to human welfare;
- better facilities for prenatal diagnosis should be developed;
- more research should be undertaken, particularly in certain areas, including prenatal diagnosis, population screening for heterozygous carriers of specific mutant genes, and the identification of environmental pollutants that might increase mutagenesis. Follow-up studies on children born after amniocentesis are also needed to assess the long term effects of the procedure, as are studies on the effects of inborn errors of metabolism in the mother or the fetus;
- medical genetic centres should set up registries of genetically determined disorders.

NEWS AND VIEWS

Marginal Basins Form by Seafloor Spreading

THE problem of the origin of the marginal basins which lie between trench-island arc systems and their corresponding continental margins has vexed Earth scientists for as long as any living geologist can remember. Of course, there has been no shortage of proposals, either for the origin of marginal basins in the context of island arc development as a whole or for the formation of single basins seen as localized phenomena. In the early part of this century, for example, the discovery within island arc systems of rocks thought to be characteristic of continents led many geologists to believe that marginal basins were continental areas which had subsided, subsidence being attributed variously to crustal warping associated with orogenic compression, subcrustal erosion, oceanization (and that very recently), and many other processes. On the other hand, Wegener, who quite naturally viewed the world in terms of continental drift, saw marginal basins as the extensional spaces left in the wake of moving land masses.

Not surprisingly, the conflict between the various hypotheses for the origin of marginal basins stood little chance of resolution until much more information about the basins themselves became available; and the acquisition of hard data only became possible with the development of reliable marine geophysical techniques during the late 1950s and early 1960s. Then, for example, seismic refraction studies showed the crust beneath marginal basins to be similar to that of normal ocean basins and bathymetry indicated that the basins have oceanic depths which are intermediate to normal. These and other discoveries clearly required the rejection of all hypotheses describing marginal basins as essentially continental; and with the field thus narrowed the way was open to a more detailed comparison between marginal basins and the wider ocean floor the origin and evolution of which were coming to be seen in terms of the now-familiar processes embodied in the new global tectonics.

The next important step was the deduction from a wide variety of geological and geophysical evidence that marginal basins must be created by crustal extension. Apparently it took some time for this conclusion to follow from the available data, for as late as 1969 some workers were attempting to explain the high heat flow in some marginal basins by models which did not involve extension. But in an article which has now become an important landmark in the subject, Karig (*J. geophys. Res.*, **76**, 2542; 1971) convincingly pressed the case for crustal extension from a consideration of all geological and geophysical data then available. In the process, he found it convenient to divide marginal basins into two natural groups—the first involving those basins in which crustal extension is currently active (Tonga, Kermadec, Mariana, Bonin and New Hebrides) and the second comprising those in which extension has ceased (the rest). The inactive marginal basins were then further divided into those with high heat flow and those with normal heat flow, the difference being essentially one of age. Karig supposed that once activity in a basin had ceased the crust would cool, so that, by implication, the basins with high heat flow should be younger.

It follows from this view that marginal basins differ principally only in their stage in history, although Karig admitted that the case for extension in the now inactive basins is based primarily on similarities with active basins rather than the result of direct evidence. But accepting an extensional origin for all basins, Karig went on to propose a model for the extension which involved a thermal diapir of mantle material rising buoyantly from the shear-heated upper surface of the downwelling lithosphere. At the same time, Packham and Falvey (*Tectonophysics*, **11**, 79; 1971) were proposing the injection of mantle material close to island arcs, resulting in a form of asymmetric seafloor spreading. Unfortunately, neither Karig nor Packham and Falvey had access to one vital piece of information—an adequate set of magnetic data from a marginal basin.

As Hayes and Ringis show on page 454 of this issue of *Nature*, such data are now available for the Tasman Sea (inactive and with normal heat flow in Karig's classification) and indicate symmetric magnetic anomalies which correspond to part of the global pattern of Cainozoic lineations. In short, the magnetic evidence together with seismic and bathymetric data demonstrate convincingly that the Central Tasman Sea formed between 80 and 60 million years ago by a process of symmetric seafloor spreading almost identical to that now taking place in the principal oceans of the world. In view of Karig's case for the similarity of marginal basins, even this one example strongly suggests that most, if not all, other marginal basins will ultimately be found to have formed in like manner.

P. J. S.

Selecting Sequences

READERS will recall the experiments on evolution in the test tube in which the template dependent RNA polymerase from Qβ infected *Escherichia coli* replicates Qβ RNA which evolves in response to the metabolic challenges provided by Spiegelman and his colleagues. In particular, when the products are selected for rapid growth, the greater part of the original genome—containing genes not immediately concerned with replication *per se*—are jettisoned and a much shorter molecule is produced. This too can evolve in response to changes in the reaction mixture, and it becomes interesting to ask what detailed molecular changes occur.

Mills *et al.* (*Science, N.Y.*, **180**, 916; 1973) have now provided the first steps to an answer to this problem by sequencing the 218 residue molecule which they call MDV-1. The methods used in this operation involve several clever dodges worthy of comment. MDV-1 replicates by way of a two stranded intermediate and both the plus and minus strands are copied though apparently with unequal efficiency. It is thus possible to make a mixture of both strands which can be annealed to give a mixture of the plus strand and a 1:1 complex of the plus and minus strand; these can then be separated by polyacrylamide/gel electrophoresis and studied separately.

First, the plus strand was completely degraded with both ribonucleases T_1 and A and the two sets of oligonucleotides were sequenced. This was helped by the fact that the 2′ or 3′ terminal phosphate is derived from the α phosphate of the ribosetriphosphate incorporated in the nearest 3′ neighbour position. Because it was possible to grow molecules in a medium with just one of the triphosphates labelled in this position with ^{32}P, it was easy to detect the residues in the oligonucleotides with this nucleotide next to them in the 3′ position. In this way the nearest 3′ neighbours of all bases can be determined. This in itself gave most of the information necessary to sequence the oligonucleotide fragments, and a complete catalogue of the sequenced T_1 and A digest fragments from the plus strand was thus obtained. The method was then applied to the 1:1 complex of both strands and a combined catalogue for both was found. It was then a simple matter to subtract the plus strand catalogue from the combined catalogue to obtain that of the minus strand.

The nearest neighbour information together with partial overlaps between products obtained from the two sorts of enzyme and the fact that the plus and minus strand fragments were complementary enabled the fragments to be assembled into sixty-six extended sequence blocks, and the problem remained to place these blocks in the correct order. The nearest neighbour information severely restricted the possibilities but did not point to a unique solution. To eliminate ambiguities it was necessary to obtain information concerning the order in which the blocks occurred along the chain. This was accomplished by exploiting the ability of polyacrylamide gels to resolve RNA according to molecular weight. The replication system was set up to produce only minus strands and aliquots were removed and replication quenched at different times after initiation. This resulted in a set of partially completed molecules each of which started at the 5′ end. These were pooled and resolved on polyacrylamide gels. The resulting distribution was divided into twenty-two fractions and each was degraded by ribonuclease and fingerprinted. It was thus possible to test each fraction for the presence or absence of relevant enzymatic digestion products. If an oligonucleotide was absent it was clear that it must occur further down the chain than the point at which the RNA in that particular fraction was terminated. In this way it was possible to determine the order of the extended sequence blocks along the chain and to assemble them into a unique sequence.

The payoff of these elegant experiments comes from a close inspection of the final sequence. This was found to contain several complementary runs of nucleotides capable of forming helical regions. These helical regions tended to occur in positions along the chain which are resistant to enzymatic digestion. The authors point out that if one strand has such potential structure so must the other and that the two strands are, in a sense, mirror images. They also observe that if certain nucleotide sequences are favoured by the selection pressures which produced MDV-1, there would be a tendency for them to exist in both strands. Once this occurs there must necessarily be complementary regions in both chains leading to the possibility of secondary structure. Thus secondary structure in such molecules must be seen as a natural consequence of selection for molecules

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Immunochemistry of Hepatitis B Antigen

THE discovery of hepatitis B antigen has provided a valuable laboratory test for potential infectivity of blood and other material, but, in spite of considerable research, the biological nature of the antigen remains unknown. Morphological studies of the antigen by electron microscopy after negative staining revealed a plethora of structures including small spherical 20 nm particles. Some of these particles have several features in common with negatively stained virus particles and the finding of about 5% RNA in partially purified antigen (Jozwiak and colleagues, *Nature new Biol.*, **229**, 92; 1971) suggested a close affinity between the antigen and animal RNA viruses. The failure to confirm so far the presence of RNA in hepatitis B antigen, however, has led to several alternative proposals on the nature of the antigen.

The view that the antigen is composed of normal host-coded material such as serum proteins, assembled through the stimulus provided by infection with hepatitis B virus, and the background of genetic factors has led Blumberg and his colleagues (*J. exp. Med.*, **134**, 3205; 1971) to suggest that the antigen is a unique infectious agent which has the characteristics of an inherited serum protein polymorphism (*Nature new Biol.*, **234**, 226; 1971). Another view is that the antigen is a specific protein or lipoprotein produced by liver cells infected with hepatitis B virus. But the most likely proposition is that the antigen represents excess virus coat material, partly in the form of aggregates of protein subunits, and that the inner core of the 42 nm particle may be the infectious agent.

Biochemical dissection of the antigen now seems to provide further clues. Burrell and his associates report in *Nature New Biology* next Wednesday (June 27) the finding of significant amounts of carbohydrate in the 20 nm antigen particles. They consider it possible that either the carbohydrate is necessary for maintaining the structure and functional integrity of the antigenic determinants or that the carbohydrate itself constitutes an important antigenic determinant. This raises two intriguing possibilities. First, the carbohydrate might have a novel haptenic specificity which is either virus-coded or virus-induced host cell-coded, or, second, the carbohydrate (and indeed some lipoprotein components) might simply be derived from the host cell membranes as the mature virus particles are released by budding.

The incorporation of host specific antigenic material in the envelope of the virus does not alter the specificity of the viral antigens nor the biological properties of the virions. There may, however, be some antigenic similarities between the virus carbohydrate hapten and the carbohydrate antigens of normal cell surfaces, or between the virus protein and the protein moiety derived from the host, leading to the establishment of partial tolerance because of the antigenic similarity between hepatitis B antigen and "self" antigens. Tolerance may play a part in establishing the persistent carrier state. Another possibility is that antigen specificities which consist partly of host-derived material and partly of virus-derived material may elicit antibodies against such antigen complexes which in turn will react with the host cells, especially with virus infected cells. Such a mechanism may operate in the immunopathogenesis of liver damage in acute viral hepatitis (Farrow *et al.*, *Br. med. J.*, **2**, 693; 1970) and in chronic liver disease associated with hepatitis B infection (Zuckerman, *Immunopathology*, **6**, Schwabe, 1970, p. 436).

containing specialized sequences. The authors see this secondary structure as a phenotypic expression of the primary nucleotide sequence and it may be that the properties of these phenotypes in turn provide extra factors that may play their part in the selection of these evolving molecules. Finally, Mills *et al.* intimate that they have sequenced a mutant of their MDV-1 molecule subjected to different selection pressures.

From a Correspondent

PROTEIN SYNTHESIS

In Praise of Controls

from our Molecular Biology Correspondent

THE history of haemin as a regulator of haemoglobin synthesis has transmogrified itself into an absorbing moral fable. When it was discovered that the output of protein by a rabbit reticulocyte system was heftily stimulated by the addition of a *soupçon* of haemin, the scheme of regulation that forthwith leapt unbidden to the mind of molecular biologists, nourished on a lifelong diet of feedback, fell so pleasingly into place in the canon, was so close indeed to the way they would themselves have designed it, as to exclude all doubt of its validity. In such a situation it might have seemed wanton to jeopardize so satisfying a conceit by doing one experiment too many. That experiment has now, alas, been done and in several laboratories at once, and the result is a true Huxleian tragedy — a beautiful and virtuous hypothesis butchered by a gang of ugly facts.

The idea, of course, was that globin, accumulating apace in consequence of stimulation by free haemin, would bind progressively more of this haemin, thereby inhibiting further globin synthesis until new haemin was added to the pool. Mathews, Hunt and Brayley (*Nature new Biol.*, **243**, 230; 1973) have shown, however, that haemin stimulates equally the translation of extraneous messengers in the rabbit reticulocyte system. They have tried this experiment with another globin messenger, from the mouse, with lens crystallin messenger and with a viral RNA. The proteins that were synthesized were fractionated by gel electrophoresis, and were not globin, except when a globin messenger was used, as judged by their electrophoretic mobilities and their autoradiographic tryptic peptide maps.

In the case of EMC virus RNA the control of translation by haemin was less marked, but this is very likely a consequence of reluctant and brief initiation by this messenger and its effect in inhibiting endogenous messenger activity.

The same conclusions were in fact taken a step further by Beuzard, Rodvien and London (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 1022; 1973). In the first place they separated the freshly synthesized proteins from intact reticulocytes by ion-exchange chromatography, and found that haemin engendered essentially the same degree of stimulation on the synthesis of haemoglobin, which makes up the overwhelming bulk of the red cell protein, and that of carbonic anhydrase. They next proceeded to a totally unrelated system, ascites tumour cells, and found that here too the translation of both the endogenous messenger and of added rabbit globin messenger was stimulated by addition of haemin. The effect is not on the initial rate of the synthesis, for the build up of incorporation with time is more or less the same regardless of the presence or concentration of haemin (at least up to a limiting value), but rather on the length of time during which synthesis is sustained, so that the stimulating effect becomes apparent only after some minutes.

Yet another independent demonstration of the lack of specificity of the haemin comes from Lodish (*ibid.*, 1526), whose concern was with the synthesis of the proteins of the red cell membrane, a problem of interest in a number of connexions. The same patterns of amino acid incorporation were found whether the synthesis was observed in whole reticulocytes, or in lysates prepared by cautious procedures, that do not evidently provoke the release of membrane-bound polysomes into the solution. Evidently therefore the membrane proteins synthesized in these cells are not made predominantly on membrane-bound ribosomes (in contrast with extracellular proteins, which are synthesized on membranes for instant export as they come off the assembly line). In fact only three of the fifteen or so principal identifiable membrane proteins, as recognized by electrophoretic analysis, turn out to be significantly labelled, and the labelling patterns indicated that one of them was perhaps a precursor of another. This was confirmed by examination of the tryptic fingerprints of the two products, and by inhibition of the transformation of the one into the other by an active-site reagent specific for serine proteases. Thus only two of the principal membrane proteins are synthesized at the reticulocyte stage, and their molecular weights are estimated by detergent-gel electrophoresis to be about 53,000 and 33,000. The preponderant membrane protein species, therefore, especially the high-molecular-weight spectrins, and the blood-group antigen glycoprotein, are synthesized at an earlier stage of development. As to the effect of haemin, this is just as important for the synthesis of the membrane proteins as for the globin.

All this does not, of course, deprive the function of haemin in this role of its interest, even though it is presumably not implicated in protein synthesis in most other cells. Mathews *et al.* advert to some observations which indicate that the haemin operates by binding to and inactivating an inhibitor of synthesis. Darnborough *et al.* (*J. mol. Biol.*, **76**, 379; 1973) have, moreover, just come up with some compelling evidence that initiation in the reticulocyte system proceeds in a rather different manner to that of most tacit assumptions. They find that a complex is first formed between formylmethionine–tRNA and 40S ribosomal subunits. This complex then takes up messenger and the 60S subunit, and synthesis now begins. It appears that in the absence of haemin, the concentration of the 40S-tRNA complex runs down rapidly. It seems, therefore, that the mysterious inhibitor intervenes at this point, unless haemin is present. Whether the action of the haemin is purely adventitious is not yet, of course, clear. It is, incidentally, interesting that double stranded RNA, which is a strong inhibitor of initiation of protein synthesis, also according to Darnborough *et al.* interferes with the build up of the tRNA-40S complex. Moreover, Kaempfer and Kaufman (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 1222; 1973) have shown that it works by binding to and inactivating an initiation factor, IF-3 (though their inference is that initiation normally occurs on a base-paired segment of the messenger RNA).

SEED RESEARCH

New Directory

THE publication of a *Directory of Seed Research in Britain* (obtainable from the Scottish Horticultural Research Institute, Invergowrie, Dundee DD2 5DA) should improve communications and reduce unnecessary duplication of research effort. It covers not only agricultural and horticultural crops, but also trees, weeds and wild species. Seed production, processing, quality, storage, dormancy, germination and seedling establishment are among the topics included. The compiler—Dr T. W. Hegarty—has divided the directory into four main sections: (1) an alphabetical list of research establishments; (2) a list of research interests, arranged under name(s) of the individual or group concerned within each institution; (3) a name index; and (4) a subject index.

POLYTENE CHROMOSOMES

Selective Replication

from our Molecular Genetics Correspondent

FORMATION of the giant polytene chromosomes of the salivary glands of *Drosophila* takes place by several successive replications of the diploid genetic material. By the time the final number of nine replications has been achieved, each polytene chromosome has more than one thousand times its usual DNA content, for each successive replication doubles its amount of DNA. But some sequences suffer more doublings than others; a comparison of the amount of DNA in the euchromatic arms of the polytene chromosomes with the DNA content of the chromocentre which is formed by the aggregation of regions of heterochromatin shows that the more condensed heterochromatic DNA does not keep pace with the euchromatic sequences; instead of eight to nine doublings it is replicated at most only a very small number of times.

Prompted by this split in the control of replication in salivary gland cells, Spear and Gall (*Proc. natn. Acad. Sci., U.S.A.*, **70**, 1359; 1973) have examined the replication of the sequences which code for ribosomal RNA. The two hundred or so genes which specify ribosomal RNA are clustered on the sex chromosomes at or close to the site where the nucleolar organizer forms to synthesize ribosomal RNA. This region, although itself replicated during the formation of polytene chromosomes (as shown by incorporation of ^{3}H-thymidine) and active in the transcription of ribosomal RNA molecules, is surrounded by the heterochromatin which is replicated to a lesser extent and is not transcribed at all.

Control of replication in polytene *Drosophila* cells identifies three types of sequence. In a previous article, Gall, Cohen and Polan (*Chromosoma*, **33**, 319; 1971) used hybridization with satellite DNA to show that these sequences—a major part of the heterochromatin—appear to replicate hardly at all compared with the extensive polytenization of sequences contained within heterochromatin. Spear and Gall now show that ribosomal RNA genes fall into neither of these categories, but are controlled independently.

Cells of *Drosophila* which have the XO constitution (that is an X chromosome and no Y chromosome) have only one nucleolar organizer, whereas female cells of the XX class have two nucleolar organizers. By measuring the extent of hybridization with ^{3}H-rRNA of DNA extracted from these two types of diploid cell, Spear and Gall found that the content of rDNA is proportional to the number of nucleolar organizers. In one strain of *D. melanogaster* they found that XO cells have 0.26% and XX cells have 0.47% rDNA; in another strain the XO cells have 0.24% and the XX cells have 0.37% rDNA. This shows that a nucleolar organizer forms at each cluster of rRNA genes on an X chromosome, the two clusters in XX cells presumably being identical.

But very different results were obtained when the DNA used for hybridization was extracted from polytene cells of the salivary gland. In both strains of *D. melanogaster*, only 0.08% of the DNA corresponded to ribosomal RNA. In a polytene cell with the 1,024 eqivalents of euchromatic DNA produced by nine doublings of the diploid content, this proportion corresponds to about 150–200 times the amount of ribosomal DNA in a diploid cell. This would be generated by some six or seven doublings of the genes coding for ribosomal RNA.

But the control of replication of the ribosomal RNA genes is not merely set at a lower level than that for euchromatic DNA. In both XO and XX cells, the rDNA content of polytene nuclei corresponded to the same level of 0.08%. The ribosomal RNA genes have therefore doubled one more time in the XO cells to compensate for their lower starting value; the final level of rDNA in these cells is therefore independent of the number of nucleolar organizers with which their diploid ancestors started. In contrast to this behaviour, the remaining sequences of the X chromosome double only the same fixed number of times in XO cells and XX cells.

Ribosomal genes of these cells are therefore not only under a control independent from that of euchromatin and heterochromatin but also achieve a fixed final level (relative to the number of doublings of euchromatin) instead of doubling a certain number of times. That the control of ribosomal RNA genes is especially important in the cell has been shown also by the phenomenon of magnification discovered by Ritossa, in which mutants with a reduced number of ribosomal RNA genes seem to be able to increase the number in germ line cells so that the wild type situation is restored. Both lateral extension of the number of genes in one chromosome and the multiplication of the number of chromosome rDNA regions are therefore utilized to maintain a specific content of ribosomal RNA genes in different circumstances.

BACTERIOLOGY

Hospital Bugging

from a Correspondent

HEXACHLOROPHANE has been under attack because it may lead to neurological disorders if used in large amounts for washing babies, and it has been withdrawn from most germicidal soaps. But hospital staff still find it valuable for hand washing and preoperative

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Haemoglobin Prediction Verified

THE survey by Perutz of abnormal human haemoglobins has revealed that whenever the mutation involves only an amino acid on the outside of the molecule there are no aberrations in the functional characteristics. An exception to this rule was the β-chain variant, haemoglobin Seattle, which was reported to have glutamic acid in place of alanine at position 76. The side chain in this position is on the surface, and not apparently involved in any intramolecular interactions. Thus either Perutz's scheme of the structure and function relation contained a flaw, or the fingerprinting analysis as published was in error.

The second explanation has now been proved correct. Kurachi *et al.*, writing in *Nature New Biology* next Wednesday (June 27), report the results of a re-examination of the deviant peptide component in the fingerprint map. They find that rather than a glutamic acid for an alanine at position 76, haemoglobin Seattle has aspartic acid for alanine at position 70, which in Perutz's model is in contact with the haem group. The functional disturbance which this substitution induces is not large, the haem-haem instructions and Bohr effect being normal. There is, however, a decrease in oxygen affinity, and an enhanced tendency towards oxidation to methaemoglobin.

In an accompanying letter, Anderson, Perutz and Stamatoyannopoulos describe the structural consequences of the replacement. The difference electron density map reflects the substitution itself, and in addition a displacement of another aspartate side chain and a small disturbance of the haem group, which is tentatively identified in terms of an alteration in the tilt. In the deoxy-form the β carbon and the carboxyl group of the anomalous aspartate side chain are close to two different methyl side chains of the haem. One of these contacts would be lost in the transition of the conformation to that of the oxygenated form.

It is not obvious why these distortions would have the effect that they do on the properties. Anderson *et al.* conjecture that the negative carboxylate group may polarize the haem sufficiently to affect the oxygen affinity, and the increased negative charge could also make it more susceptible to oxidation.

scrubs. It now seems, however, as if hexachlorophane does not give adequate protection against Gram-negative organisms, and thus it can be classified with other cleansers which have been giving hospital workers a false sense of security. For example, Gram-negative organisms have been shown on occasions to be spread in hospitals by cloths and mops—the cleansing solutions being relied on to clean them are inactive against these organisms.

Gram-negative organisms are increasingly becoming a problem in hospital-acquired infections. In the past the most feared organisms were the Gram-positive staphylococci and streptococci which became resistant to the antibiotics in common use. Hexachlorophane has undoubtedly diminished this worry, but is contributing little to the Gram-negative problem.

Two workers at the University of Bergen School of Medicine (Bruun and Solberg, *Br. med. J.*, **2**, 580; 1973) have studied the effect of hexachlorophane and of ordinary bar soaps on the colonization of the hands by Gram-negative organisms. The bacterial hand flora of nursing staff in one medical and two surgical departments were examined for *Staphylococcus aureus* and the Gram-negative organisms *Pseudomonas aeruginosa, Proteus* and lactose-fermenting coliforms. Two sets of samples were obtained, with a period of 6 months between them during which one group washed their hands with bar soap only, one with bar soap for 3 months and 3% hexachlorophane for 3 months, and the third with hexachlorophane only. Carriers of Gram-negative organisms were followed with regular sampling.

The results confirm again that hexachlorophane reduces the carriage of *Staph. aureus*. But when all the counts obtained during the hexachlorophane period were compared with counts during the period of using soap only, there was a significantly higher isolation rate for Gram-negative bacilli in the hexachlorophane group. At the start 18.8% of the staff in the survey carried Gram-negative organisms, and it seems that 13% of these continued to carry these organisms for periods of 6 to 20 months. All these persistent carriers were in groups using hexachlorophane.

Unfortunately Bruun and Solberg say nothing about how thoroughly the hands were dried after washing, which is important because Gram-negative organisms flourish best in moist conditions. All the same, the results indicate that hexachlorophane cannot always be relied on. The authors point out that most of the persistent carriers had signs of low-grade infections of the skin of the hands for most of the investigation period, and most of the Gram-negative organisms were isolated from these infected areas.

PLANT GEOGRAPHY

Origin of Cockleburs

from our Plant Ecology Correspondent

THE variation of photoperiodic response found in related plant species from different latitudes is now well documented. One of the most informative genera is *Xanthium* (cocklebur) which has a widespread distribution, but which has an extremely complex taxonomy, chiefly as a result of its morphological plasticity. Ray and Alexander (*Am. J. Bot.*, **53**, 806; 1966) found that *Xanthium strumarium* varied in its photoperiodic response according to the latitude of its origin in North America. Plants of northern origin, for example, Montreal, Quebec, required a critical dark period of only 7.5 h to initiate flowering, whereas plants from the south, for example, Texas, required more than a 10-h dark period. This response is genetically controlled and can be regarded as an adaptation to differing climatic regimes in the North American continent.

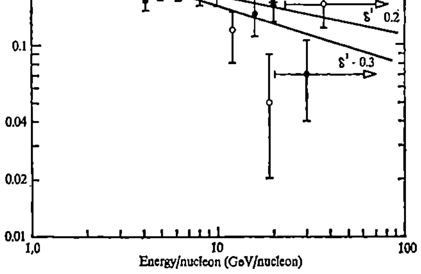
McMillan (*ibid.*, **57**, 881; 1970) has since shown variation in photoperiodic response with latitude in Texas, though in Mexico this was not found to be the case. There, considerable variation occurred within a given latitude, which McMillan explains by reference to climatic variability resulting from irregular topography.

Xanthium is a genus of annual plants characteristic of open habitats. It has invaded several areas of the world such as India and Australia as an aggressive weed. Recently McMillan (*Nature*, **240**, 485; 1972) attempted to trace the geographical origins of the aggressive Indian populations by examining their photoperiodic responses as well as some chemical characteristics. He concluded that the aggressive form was the product of hybridization between indigenous genotypes and a Caribbean taxon, the fruits of which could have been imported in American cotton seed.

McMillan has now turned his attention to the *Xanthium* populations of the Pacific Islands (*Am. J. Bot.*, **60**, 277; 1973), which are regarded as adventives, probably of American origin. He collected seed from plants in Tahiti and Oahu and also from several areas of Mexico at similar latitudes for comparison. Plants grown from the island seeds had a critical dark period requirement of between 10.75 and 11 h. Mexican material showing greatest similarity in response came from the north-western coastal plain in Tamaulipas. Most other Mexican plants had shorter dark period requirements. When grown together at Austin, Texas, in experimental conditions, the populations were shown to differ in a variety of other physiological and morphological characteristics, but the similarity of their photoperiodic requirements gives strong support to the contention that the *Xanthium* populations of the Pacific Islands originated in coastal Mexico. Degener's theory that Hawaiian *Xanthium* was introduced from California now seems most unlikely, because Californian plants have critical dark period requirements varying from 8 h in the north to 9.5 h in the south. The island populations evidently originated in more southerly latitudes.

Galactic Escape and Cosmic Electrons

THE implications for cosmic electrons of the discovery that the escape of cosmic rays from the Galaxy is apparently dependent on energy are examined in next Monday's *Nature Physical Science* (June 25) by Silverberg and Ramaty. They come to the conclusion that the effects of the loss of electron energy by the Compton and synchrotron processes on the electron energy spectrum will be much less than if the "lifetime" in the Galaxy of the electrons is constant at all energies.



The known variation of the ratio of light nuclei ($3 \leqslant Z \leqslant 5$) to medium nuclei ($6 \leqslant Z \leqslant 8$) with energy is shown in the diagram. The lighter nuclei which are produced by fragmentation of the heavier nuclei during their passage through the Galaxy are less in evidence at higher energies, suggesting that an energy-dependent loss process is at work (or, alternatively, that considerable fragmentation takes place in the sources of cosmic rays).

Assuming that the energy dependence of the loss of electrons from the Galaxy is described by similar parameters to those which describe the effect for nuclei, Silverberg and Ramaty show that the change in electron spectral index resulting from the electron energy loss processes is unlikely to be large at electron energies below several hundred GeV.

GRAVITATIONAL RADIATION

Magnetic Correlation?

from a Correspondent

THE chief evidence for associating the signals recorded by Weber's gravitational wave detectors with an astronomical source outside the solar system has been the 12-hour periodicity displayed in published plots of event rate as a function of sidereal time. Now a wider analysis of the times of some of Weber's events has been made, and this indicates that there may also be correlations with disturbances of the Earth's magnetic field and with sunspot activity. Such correlations would suggest that Weber's detectors may be influenced by phenomena unconnected with gravitational radiation and may help to resolve some of the problems raised by Weber's observations.

The new analysis has been made by Tyson, Maclennan and Lanzerotti of Bell Laboratories (*Phys. Rev. Lett.*, **30**, 1006; 1973), and follows earlier work by Adamyants, Alekseev and Kososnitsyn in the Soviet Union. The Soviet physicists based their analysis on published times of seventeen coincident events recorded by Weber's detectors at Maryland and Chicago in late 1968 and early 1969. They found a correlation between changes of terrestrial magnetic activity and the days when Weber events were recorded and they also found a rather stronger correlation, involving a time lag of 2 days, between sunspot activity and Weber pulses. These results have generally been regarded as interesting, but of limited significance because of the small number of events analysed.

The group at Bell Laboratories was provided by Weber with data on 262 events recorded with his detector in a period of 121 days beginning in August 1969. They have searched for correlations between the daily rates of Weber's events and many other phenomena including geomagnetic activity, sunspot number, averaged earthquake amplitudes at a point between Maryland and Chicago, tidal strain in the Earth and changes in barometric pressure and in temperature. Correlations at levels above 2 standard deviations are found between the Weber data and geomagnetic, sunspot and earthquake activity; with a time lag of 8 days in the sunspot correlation. The strongest geophysical correlation found was with magnetospheric ring-current intensity and was at the level of 2.7 standard deviations. The rate of occurrence of the gravitational wave events throughout the sidereal day was also investigated and a distribution similar to those reported by Weber was obtained, with peaks occurring twice a day at the times when the detectors would be expected to have maximum sensitivity for gravitational radiation coming from the direction of the galactic centre. This correlation was at the level of 3 standard deviations.

What do these correlations imply? In the case of the magnetospheric correlations the Bell Laboratories group points out that the enhanced low frequency magnetic disturbances which are present at times of high geomagnetic activity may couple into Weber's detectors and produce some "events"; it is known that fluctuations in the magnetic fields can be correlated sufficiently closely in time over distances equal to that from Maryland to Chicago to be recorded as coincident in Weber's equipment. Weber has carried out experiments to search for magnetic coupling to his detectors, but it seems that his published tests were made with magnetic sensing equipment tuned to frequencies related to the resonant frequencies of his detectors. It may be that very low frequency magnetic fluctuations would not be efficiently detected in such tests and might penetrate the steel vacuum tanks and cause vibrations in ferromagnetic materials coupled to the aluminium bars.

It is more difficult to understand the reported sunspot and earthquake correlations, and the Bell Laboratories group does not suggest interpretations here. In fact they do not claim that their analysis indicates the prime source of Weber's events. The point they make is that, as the correlation with some geomagnetic phenomena is almost as strong as the correlation with sidereal time, at least a certain fraction of Weber's events may be caused by processes other than gravitational radiation.

As the authors indicate, however, the data used in this study represent only a small part of the body of data now collected by Weber and a more extensive analysis might give different results. Clarification of the problem may come from some of the independent experiments with gravitational wave detectors now in progress. None of the results reported to date has confirmed Weber's observations, but most of the experiments have only collected data over relatively short periods, and it may be some time before unambiguous conclusions can be drawn.

MARS

Are Ice Ages Temporary?

by our Cosmology Correspondent

AT a special seminar held at the University of Sussex on June 8, Professor Carl Sagan, of Cornell University, presented some of the evidence from the Mariner 9 photography which has led him to suggest that Mars may be in a temporary ice age at present, and that more clement conditions recur on the planet periodically. This idea has exciting implications for the prospect of finding evidence of life on the planet.

Sagan's discourse was divided into two parts; the first covered the implications of the great dust storm which was raging when Mariner 9 arrived at Mars, and which seems to be a typical pheno-

Spiral Structure and Nuclear Activity in Galaxies

THE density wave theory can explain many features of the spiral structure of galaxies, but requires a regular input of energy. One recent suggestion is that regeneration (about every 10^9 yr) is associated with gas expelled from the galaxy's nucleus. That idea has been followed up by van der Kruit, who describes in *Nature Physical Science* next Monday (June 25) a model in which a continuous cycle links nuclear activity with regeneration of spiral structure, while angular momentum transport by density waves adds to the production of nuclear energy.

Observational data which help in the development of this model have been carried out at Westerbork by van der Kruit, and reveal two important correlations. First, the relative velocity bewteen gas and the spiral pattern, $\omega_{\perp 0}$, is larger when the ratio of radius at which the rotation curve is maximum (R_m) to optical radius (R_o) is smaller. Moreover, a similar relation holds for the compression strength and this ratio; both relations are distance independent.

The Westerbork survey data provide measurements of compression strength for only fourteen galaxies, but use of the R_m/R_o relations and of van den Bergh luminosities enables van der Kruit to extend this sample to twenty-six galaxies with well observed rotation curves. Galaxies with low R_m/R_o, and thus strong compression, are relatively more luminous because of enhanced star formation.

Further observational data suggest that galaxies with low R_m/R_o also contain better developed nuclei, showing clear signs of activity. Because nuclear explosions can regenerate the density waves by gas expulsion, but these same density waves then transport angular momentum such that gas and rotational energy are fed into the nucleus, van der Kruit suggests that the observations indicate the presence of a closed cycle, both directions of which operate more strongly in galaxies with low R_m/R_o. There is, of course, some scatter in the observations, and the precise nature of the expulsive mechanism remains unknown. This work points the way to a new and interesting approach to the problem of spiral structure, rather than presenting all the answers.

menon at perihelion. The erosive power of the wind-borne dusts is impressive enough to lend credence to the radar observations from Earth which suggest that large areas of Mars are covered by thick layers of dust and would not form easy landing grounds for vehicles such as Viking. Furthermore, the streaks of light and dark coloured dust which lie in the wind shadows of many of the craters suggest that the dust has a two component structure, as well as providing an insight into the variations of the wind. Thus, it now seems almost certain that the change in shape of the dark splotches on Mars is a result of the movement of light coloured dust over the darker dust; the once favoured idea that the sometimes dramatic growth of these dark areas could be attributed to plant growth now seems untenable.

But if the exobiologists have suffered a disappointment in this regard, Sagan's other news more than made up for it. This centred on the presence of the remarkable sinuous channels which so clearly suggest the presence, at sometimes in the past, of a flowing, low viscosity liquid. The channels are concentrated near the equator suggesting that the liquid in question must be one which can exist, at times, only in that part of the planet. Only one possible liquid fits the bill—water can flow in the equatorial regions of Mars if the planet's temperature is increased slightly. A 15% increase in the heat absorbed at the poles of Mars would produce a self sustaining temperature change, because the release of carbon dioxide would increase the atmospheric pressure, making the climatic circulation which carries warm air from the equator to the poles more efficient, and preventing the CO_2 caps from reforming.

This sort of increase in absorption is by no means impossible. Sagan listed four possibilities:

- Changes in the heat produced by the Sun. This has interesting astrophysical implications which are discussed by Sagan and Young in a letter on page 459 of this issue of *Nature*.
- Changes in the obliquity of the planet's axis to at least 28°. This is consistent with an independent suggestion from planetary astronomers that the obliquity of Mars may vary, up to 34°, with a period of some millions of years.
- Polar wandering, by which the CO_2 ice could be moved into the warmer parts of the planet.
- A decrease in the albedo of the CO_2 ice by only 3%, lasting for at least 100 years.

The last idea is perhaps the most immediately attractive because the albedo of the Martian dust is only about one third of the 75% albedo of the present polar caps, and there is no doubt that large quantities of dust are frequently stirred up in the Martian atmosphere.

Mars today, then, may be in a temporary ice age. At other times, the planet may be warm enough for running water to flow near the equator, although this would be associated with melting of a permafrost layer rather than rainfall. Obviously, this raises the prospect that some form of life could evolve during the warm spells, and even that spores would survive through the ice ages to await the next warm period. As Sagan pointed out, spores can survive for very long periods on Earth, where there has hardly been the same kind of evolutionary pressure that similar organisms would experience on Mars. Such spores are triggered into activity by the presence of water, and two of the experiments on the Viking landers involve the addition of water to samples brought into the spacecraft. That prospect should certainly rouse still further interest in the Viking missions.

NUCLEAR PHYSICS

Neutrino Interactions

from a Correspondent

THE beauty of the parton model of subnuclear matter which was introduced by Feynman is its simplicity. A solid state physicist sees atoms as point-like structures in his solid; as physicists probe further the atoms are seen to have smaller point-like nuclei surrounded by electrons; on closer examination still, nuclei are found to consist of protons and neutrons bound together by the nuclear force. Then the proton and neutron are found to be composite structures themselves with point-like structures within them called partons. In the simplest form of the model these are identified with the three quarks which describe the strong interaction symmetry SU3.

The predictions of the model are beautifully described in Feynman's lecture note volume *Photon-Hadron Interactions* (Benjamin, New York). The model was constructed to describe the deep inelastic electron scattering measured at the Stanford Linear Accelerator Center (SLAC), which revealed point-like structures possessing spin inside the proton.

New data have now been presented, some of which confirm the model, some which tend to contradict it, and all of which whet the appetite for more information.

At the National Accelerator Laboratory (NAL) at Batavia a neutrino beam has been produced from the 400 GeV proton beam. In a recent *Physical Review Letters* (**30**, 1084; 1973) the first results are presented of events produced by neutrinos in spark chambers. Events with a transfer of four momentum to the nucleon of 100 $(\text{GeV}/c)^2$ are observed, corresponding to distances smaller than 10^{-15} cm; at this distance the interacting structure still appears to be a point; the number of events caused by antineutrinos is one-third of the number caused by neutrinos, in agreement with low energy data and the simple model. This is stretching the applicability of the model to smaller distances than before, and opens up a new field of physics of which more will be heard—neutrino spectroscopy.

At the Cambridge Electron Accelerator (CEA), experiments with electron-positron colliding beams have been in progress for more than a year. A recent report was given at the Washington meeting of the American Physical Society, and is soon to be published in part in *Physical Review Letters*. As has been already observed at Frascati in Italy, there is a large probability for producing events with many hadrons in the final state. According to the simple parton model, these hadrons are produced with an intermediate state of a parton-antiparton pair. Partons, if they are quarks, have spin quantum number of $\frac{1}{2}$, and the cross-section is therefore related to the cross-section for producing point-like, spin $\frac{1}{2}$, muons, by the relationship $R=\Sigma q_i^2$; where q_i is the charge of the ith parton. Because the charges on the quarks are $\frac{1}{3}$, $\frac{1}{3}$ and $\frac{2}{3}$, $R=\frac{2}{3}$. Experimentally, Frascati scientists already found $R\approx 1$, but now at CEA it is definitely confirmed that $R=4.6\pm 1.1$ at $q^2=16$ $(\text{GeV}/c)^2$, possibly rising to 7 ± 3 at $q^2=25$ $(\text{GeV}/c)^2$.

The parton model can be modified by assuming "sets" of quarks. The first modification allows "coloured" quarks (red, white and blue) and $R=2$. But this is not enough, and deeper modifications seem necessary which may destroy the model's simplicity—and will certainly change Feynman's book.

The model can also be tested by more precise measurements in a known region. Detailed measurements on the ratio of (e, p) to (e, d) scattering have been made at SLAC, and are also described in *Physical Review Letters* (**30**, 1087; 1973). These are interpreted as a ratio of (e, p) to (e, n) scattering and the neutron structure can therefore be derived. The neutron and proton, having different charges, are composed of different quarks, so that the experiment is sensitive to these details. A lower bound of 0.25 is imposed on σ_n/σ_p by the parton model. The data show that this is approached at large values of a variable $x=q^2/2MV$, and this is very hard for the simple theory to describe. One should, however, probably only expect the simple theory to describe gross features, and should expect precise experiments to show deviations.

Ageing of Clones of Mammalian Cells

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The chief purpose of this article is to review some recent work on the ageing of mammalian cells and to comment on the theoretical interpretation of the experimental findings. No claim will be made that cellular ageing is a primary cause of the ageing changes observed in whole animals; the evidence supporting such a causal connexion is inconclusive.

HAYFLICK has emphasized a possible connexion between the senescence of clones of mammalian cells maintained in tissue culture, and the ageing of members of the corresponding species[1-2]. He showed that the history of a tissue culture of human fibroblasts can be divided into three phases. His phase I corresponds to the establishment of a primary culture, his phase II to a period when vigorous growth follows each successive subculture, and his phase III to a period in which growth capacity declines until it is impossible to maintain further subcultures. These findings have been confirmed in a number of laboratories.

Hayflick reported that when fibroblast cultures are established from embryonic lung, the cells divide about fifty times before the cultures die out, but that cultures derived from adult lung die after about twenty divisions on average. There was a wide experimental scatter in these experiments, but more recent work has put Hayflick's principal conclusion on a sound statistical basis; the average number of divisions obtained declines by 0.20 for each year of the donor's life[3].

Hayflick's experiments and his tentative conclusions have been criticized on a number of grounds. It has been asserted that the limited capacity of human fibroblasts to proliferate in tissue culture reflects deficiencies of the medium rather than any intrinsic property of the cells. Supplementation of the medium, for example, with hydrocortisone[4,5] or with tyrosine[6] leads to a substantial increase in the number of doublings that occur before cultures enter phase III. Further, the Hayflick phenomenon is said not to be completely general. Chick fibroblasts, like human fibroblasts, cannot be maintained in culture indefinitely[7]. But it is claimed that a number of diploid cell lines derived from rats have been maintained in culture for well over a hundred doublings, without deterioration or obvious evidence of transformation[9]. This conclusion is disputed[10].

The dependence of the longevity of cell lines on the donor species, the cell type and the culture conditions is important. It warns us not to draw far-reaching conclusions from very limited experimental data. Hayflick's experiments do not show that humans age because their fibroblasts lose the capacity to proliferate after about fifty divisions. Hayflick's own important conclusion, that fibroblasts from adult human donors are reproducibly different from cells from embryos with respect to their proliferative capacity under a wide variety of culture conditions, is, however, not in doubt. More recently it has been claimed that size distribution of human fibroblasts in culture also varies with the age of the donor; phase II cultures derived from adult tissue are said to resemble phase III cultures derived from embryonic tissue[11].

The techniques of tissue transplantation and cell transfer permit the study of the survival and duplication of animal cells over periods longer than the life span of a single animal. The results that have been obtained in this way do not fit any single pattern. In many cases proliferation has been shown to be limited, often to a clonal life span similar to the life span of the animal. In others, tissues have continued to survive in an apparently normal state for much longer than a single life span. The nature of the tissue, the donor species, and the technique of transfer or transplantation all influence the outcome of the experiments[12].

The work of Williamson and Askonas provides a particularly clear-cut example of cellular ageing *in vivo*[13]. They used bone marrow from mice that had been immunized with a DNP-protein conjugate to repopulate acceptor mice whose immune system had been destroyed by heavy irradiation. The technique of focusing electrophoresis was then used to show that, in many cases, the acceptor mice were producing a unique antibody to DNP, presumably because a single clone of DNP-specific cells had become established.

Williamson and Askonas next attempted to carry out serial transfers of bone marrow to one generation after another of irradiated acceptors. They found that the marked clone could be transferred efficiently up to four times. After that antibody production fell off with each successive transfer and the original clone soon died out. It is estimated that the cells of the marked clone went through about ninety doublings in all.

Numerous studies on tissues taken from whole animals have shown that the total activities of many enzymes change with age. These studies, however, have not led to any important generalizations—the activities of some enzymes go down with age, but many enzyme activities are unaffected and some increase[14,15].

Studies of the specific activity and thermolability of enzymes, quantities which reflect the quality of the proteins, have given more promising results. When glucose-6-phosphate dehydrogenase (G6PD) or 6-phosphogluconate dehydrogenase (6PGD) from young cultures of MRC-5 fibroblasts is heated, enzyme activity decays exponentially, showing that the corresponding enzyme is homogeneous. The activities of these enzymes when isolated from cells in phase III, on the contrary, decay in a way that reveals the presence of up to 25% of abnormally thermolabile protein[16]. In related experiments it has been shown that the specific activity of lactic dehydrogenase isolated from cultures of MRC-5 cells in phase III is lower than that of the same enzyme isolated from cultures in phase II[17]. Gershon and Gershon have found similar alterations with age in the properties of the aldolase isolated directly from rat liver[18] and of isocitrate lyase from a nematode[19]. They interpret their results to show that the materials obtained from old animals consist of mixtures of an unaltered protein and a unique altered species.

In their experiments on human fibroblast cultures Holliday and Tarrant found[16] (1) that the proportion of thermolabile protein increases with the age of a culture (Fig. 1) ; (2) that the ratio of the proportion of thermolabile G6PD to the proportion of thermolabile 6PGD remains constant as cultures age ; (3) that treatments which arrest the growth of cells, for example deprivation of serum, do not cause the

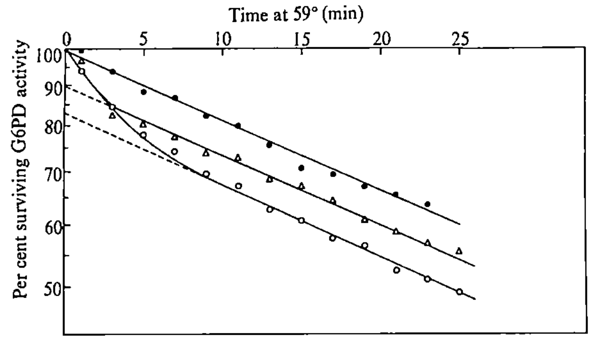

Fig. 1 Inactivation at 59° C of glucose-6-phosphate dehydrogenase obtained from young, middle aged and senescent cells. ●, Passage 22; △, passage 48; ○, passage 61. Reproduced with permission from Holliday and Tarrant[16].

accumulation of thermolabile protein ; (4) that cells grown in the presence of low levels of 5-fluorouracil, a base analogue known to induce errors of protein synthesis, age prematurely. The ageing cells accumulate thermolabile protein after a relatively few subcultures ; (5) that tissue cultures derived from a donor with Werner's syndrome already contain substantial amounts of thermolabile protein when they enter phase III after less than ten subcultures (R. Holliday, personal communication).

Holliday's results taken together with those of Lewis and Tarrant make it very probable that MRC-5 cells in phase III contain substantial amounts of protein that differ from the standard proteins present in cells in phase II. It seems unlikely that thermolabile isozymes of two independent enzymes are produced in old cultures, so aberrant protein is presumably present. These experiments, however, do not establish that protein synthesis is inaccurate. An alternative explanation is that post-synthetically modified proteins accumulate in old cells.

It is theoretically possible that the thermolabile proteins in old cells are derived entirely, or in part, from non-dividing (dead) cells and represent a protein fraction that is partially denatured or partially degraded. The enzymatic deamination of asparagine and glutamine residues, if it occurred at random positions in proteins, could well produce the observed results. A mechanism of this kind is not excluded by control (3) because serum-deprived cells, unlike old cells, might not contain the relevant degradative enzymes in an activated form. The experiment with 5-FU is not conclusive because the induction of thermolabile protein by 5-FU might be an indirect consequence of cell death rather than a direct consequence of errors of protein synthesis induced by the analogue. Recent experiments by Holliday (unpublished) argue against this explanation. They suggest that the proportion of thermolabile protein in moribund cultures is less than in cultures in phase III.

If it turns out that, in old cultures, newly synthesized proteins do have incorrect sequences, it will then be necessary to decide whether inaccurate protein synthesis is responsible, or whether the cultures have accumulated a wide variety of independent mutations in the G6PD and 6PGD genes. Holliday's results on the rate of appearance of thermolabile enzymes are not easily explained in terms of somatic mutations, but they do not completely exclude such an explanation. The distinction between mutations and errors of protein synthesis could be made by studying clones derived from single cells, by studying the components of the protein synthetic apparatus *in vitro*, or by examining virus-specified proteins.

It seems likely, on theoretical grounds, that mutations accumulate in tissue cultures as they age. The evidence on this point, however, is restricted to preliminary observations by Fulder[20], who has shown that glucose-6-phosphate dehydrogenase mutants are more common in old than in young human fibroblast cultures. There is a good deal of evidence that the frequency of gross abnormalities in chromosome structure increases with age in cells taken from whole animals[21].

Old tissues differ from young ones in another way that implies a difference in their macromolecular composition. There are many indications that the induction of enzymes in old tissues is abnormal. The induction of tyrosine aminotransferase in old tissues, for example, is slower than in young, although the same rate of synthesis is attained eventually[22]. It is not possible, at present, to decide whether such differences in the rates of induction originate in changed nucleic acids, in changed quantities of the various proteins that control gene expression, or in a change in the accuracy with which control proteins are synthesized.

Theoretical Considerations

(*a*) Organelles. The various theories of clonal ageing are usually supposed to explain modifications of nuclear DNA or cellular protein that occur in cultures as they age. Such theories are equally applicable, however, to changes in the corresponding components of the mitochondria, because mitochondrial DNA and some mitochondrial proteins are synthesized by more or less independent mitochondrial systems. It is possible that mitochondria are more susceptible to the effect of mutation or errors of protein synthesis than are the cells in which they are located. If so, the ageing of the cells might be a consequence of the deterioration of their mitochondria. In the blowfly, ageing is accompanied by the uncoupling of oxidative phosphorylation, but the nature of the molecular changes that occur in the mitochondria has not been determined[23].

(*b*) Subcultures and cell divisions. The terminal stage in the senescence of a tissue culture is usually identified as the point at which it becomes impossible to split a sample of cells into two equal portions and to regenerate the original number of cells from one of the halves. But each sub-clone does not go through a number of divisions equal to the number of subcultures, for a proportion of non-dividing cells is always present in fibroblast cultures. The relatively small scatter in the number of subcultures obtained before death is, in part, a consequence of an averaging procedure that is implicit in the design of the experiment. As the cultures get older, the proportion of healthy cells decreases, so those cells that can still divide must go through an increasing number of divisions, on average, before the total cell number doubles. This, so to speak, compresses the period of senescence into a few subcultures, although the scatter in the number of cell divisions that can occur before the death of a sub-clone may be much larger. A similar compression may occur *in vivo*.

The heterogeneity of the cell populations must be borne in mind when interpreting the results of biochemical experiments on ageing cultures. The differences being average properties of young and old cultures may give more information about cells that have ceased to divide than about the cells which are responsible for continued growth. It is important to distinguish progressive changes that accompany the ageing of mitotically competent cells from catastrophic changes that follow cell death (release of lysozomal enzymes, and so on).

(*c*) Dilution theories[23]. The simplest of all the mechanisms that have been proposed to explain clonal ageing, the dilution of an essential and irreplaceable component of the primary cells, is ruled out on numerical grounds—the molecules would be diluted out long before the fiftieth division. A closely related mechanism, the dilution of an essential component that is synthesized at a rate that just fails to keep pace with cell division (the mitochondria, for example), is compatible with what we know about ageing, but does not seem very plausible. The simple accumulation of damage also fails to account for the ageing of dividing cells, for damaged molecules would be diluted out by newly synthesized material. This last argument does not hold for non-dividing cells, and it is possible that the ageing of neurones, for example, is due to postsynthetic damage to DNA, proteins, or other cellular components.

(*d*) Molecular clocks and programmed ageing. It has been suggested that the ageing of the individual, far from representing an inevitable consequence of the limited accuracy of biological processes, has a positive adaptive value for the species. Thus, well-defined mechanisms may have evolved that guarantee senescence at an appropriate time. These ideas lead naturally to the notion of a molecular clock, a device that counts cell divisions (or elapsed time) and triggers an irreversible ageing process when the optimal life span is approached[25].

Very little is known for certain about the factors determining reproductive life span and longevity in different species. It is even uncertain that ageing is a significant phenomenon in wild populations ; it is said that few animals survive long enough to be able to age. It is not clear that senescence has a positive adaptive value, so it seems unwise, in the absence of experimental evidence, to take for granted the idea of "programmed obsolescence" in man and other mammals[26].

The one great merit of the molecular-clock theory is that it accounts readily for the phenomenon of transformation. If cells are programmed to age, a mutation that interfered with the programme could make them "immortal". Similarly, a transforming virus might interfere with the natural ageing programme. We shall see that the phenomenon of transformation can be explained only with some difficulty on the basis of error theories.

(*e*) Elementary protein error theory. There are two steps in protein synthesis involving discrimination between related molecules. Each activating enzyme must select a unique amino acid and load it onto an appropriate tRNA. A codon of messenger RNA must pair with the anticodon of an appropriate tRNA, but not with any inappropriate anticodon. Each of these processes is associated with a small but non-zero error frequency. Errors in RNA synthesis could also decrease the fidelity of protein synthesis.

Because the proteins that are part of the protein-synthetic apparatus are synthesized in the same way as other cellular proteins, errors in protein synthesis could be self-propagating[27]. Suppose we started with an error-free first-generation protein synthetic apparatus and used it to synthesize activating enzymes, ribosomal proteins, and so on. The second generation protein synthetic apparatus would certainly contain some errors. If we used this error-containing second generation protein synthetic apparatus to synthesize more activating enzymes, and so on, they would contain even more errors.

If the cycle discussed above was repeated many times, each successive generation of protein synthetic apparatus being used to synthesize the components of the next generation, one of two things would happen. Either the error-frequency would converge to a stable non-zero value, or it would diverge. In the former case, no ageing phenomenon would occur, but in the latter the error frequency would, sooner or later, become so large that the cell could no longer function.

When I first discussed this problem I thought that in the absence of cellular selection, the error frequency of protein synthesis would certainly diverge. I no longer believe this to be the case. It is possible that a protein synthetic apparatus containing a relatively small number of errors might be able to synthesize a new protein-synthetic apparatus containing fewer errors. This must have been true at some stage in the evolution of the genetic apparatus, unless cellular selection became important very early. The existence of enzymes that scavenge error-containing proteins makes it more plausible that a small, stable error frequency can be maintained[28].

In only one case is there direct experimental evidence that the frequency of errors of protein synthesis increases rapidly and leads to cell death. The *leu*-5 mutant of *Neurospora* synthesizes a temperature-sensitive leucine-activating enzyme which, at higher temperatures, allows other amino acids to substitute for leucine[29]. At low temperatures protein synthesis proceeds more or less normally. The *leu*-5 strain, unlike the wild-type, senesces when maintained at 35° C.

Lewis and Holliday have shown that the accuracy of protein synthesis in *leu*-5 falls slightly when this mutant is shifted from 25° C to 35° C, and then remains almost constant for a considerable time. After about 70 h the cells age rapidly, and at the same time the thermolability of glutamic dehydrogenase increases, while its specific activity falls dramatically. The "error-catastrophe" hypothesis fits all of Lewis and Holliday's extensive observations on this system[30].

There is, as yet, no experimental evidence concerning the mechanism of senescence in fungi that age spontaneously in nature, for example, *Podospora*. We have already seen that the origin of thermolabile protein in ageing fibroblast cultures is unclear. If an error catastrophe is the cause of ageing in non-mutant fungi or in mammalian cells in culture, this can only be established by the measurement of the fidelity of protein synthesis.

(*f*) Error theory—more general models[31]. Holliday and Tarrant have remarked[16] that it may not be possible to separate the contributions to cellular ageing caused by errors of protein synthesis from those due to the accumulation of somatic mutations. Errors of protein synthesis must occasionally lead to the formation of "mutator" DNA polymerase molecules which replicate DNA inaccurately. Conversely, some mutations, for example, the *ram* mutations in *E. coli*[32], must reduce the fidelity of protein synthesis. Thus inaccurate protein synthesis and inaccurate DNA synthesis are coupled phenomena.

If the coupling between different causes of ageing is sufficiently strong, we cannot use a conventional description in which the deterioration of one process, say, protein synthesis, is thought of as primary and the cause of the subsequent breakdown of other processes such as DNA replication. Instead we must use a more general description in which the fidelity of each of a set of significant processes is dependent on the fidelity of some or all of the others.

A theory of this kind forms a natural generalization of the simple protein-error theory. It retains the central idea of positive feedback—the greater the number of errors that have accumulated in the macromolecular constituents of the cell, the faster the accumulation of further errors. At the same time it permits one to consider the effect of mutations, relaxed control processes, faulty membranes, and so on, in parallel with errors of protein synthesis, without assigning priority to any one process.

The coupling between different contributions to ageing must also be important in the senescence of whole animals. The deterioration of extracellular components (collagen, say) is likely to change the intracellular environment in such a way as to reduce the fidelity of macromolecular synthesis. The inaccurate synthesis of proteins would surely have important effects on the condition of extracellular components. It follows that extracellular and intracellular mechanisms of ageing are coupled. I believe that the theoretical and experimental analysis of strongly coupled deteriorative processes will do much to clarify our ideas on the nature of mammalian ageing.

(*g*) Advanced mathematical methods. If errors of macromolecular synthesis are established by experiment to contribute to ageing, a more sophisticated mathematical treatment of the subject will be justified. The overall error frequencies and mutation rates which we have discussed will need to be replaced by error matrices, which specify the probability that any given amino acid A_1 (or nucleotide, N_1) is replaced erroneously by a different amino acid, A_2 (nucleotide N_2). It is also likely that simple treatments which utilize difference or differential equation will be replaced by a more general stochastic theory.

(*h*) Transformation. Mammalian cells in tissue culture are transformed by certain viruses, by chemical carcinogens, or spontaneously to permanent or transformed lines which can be subcultured indefinitely. It seems unlikely that DNA replication or protein synthesis is made more accurate by each of these modes of transformation. Thus, at first sight, the phenomenon of transformation seems incompatible with error theories of clonal ageing.

In fact, the situation is more complex. The switch from a "spanned" culture to one that is "immortal" does not necessarily signify a qualitative change in other cellular properties. An elementary mathematical result from the theory of branching-chain processes shows that if cells continue to divide and to produce, on average, more than one viable descendant at division, then cell cultures are potentially immortal. On the other hand, if each cell sooner or later comes to produce on average less than one mitotically competent descendant, the cultures inevitably die out. Thus a "spanned" culture would be made "immortal" by any change that increased the mean number of viable descendants sufficiently. It is important to note that this result is completely independent of the mechanism which leads to the increase in the number of viable descendants.

Consider first a very artificial situation, a culture of *E. coli* growing while exposed to a source of high-energy radiation. Suppose that each cell accumulates a lethal mutation in the time $t_{1/2}$ with probability 0.5. If cells are maintained under conditions which permit doubling in a time less than $t_{1/2}$, cultures are "immortal". Otherwise, they are "spanned". A small change in the medium could, therefore, "transform" *E. coli* in these rather special conditions.

This result cannot be applied directly to error theories of ageing, but it does suggest the sort of explanation of transformation that is needed. It must be postulated that an increased mitotic rate or the escape from contact-inhibition provides a protection against the accumulation of errors because, as in the simple example, it permits more cellular selection. This would be true if, for example, the rate of accumulation of errors (in proteins or nucleic acids) includes a term that is linear in the time (for example, a term describing errors occurring in non-dividing cells) while selection is entirely of a cellular type and occurs only through cell division.

A theory of this kind would be complicated and its predictions would depend crucially on the details of the postulated model of error accumulation, for example, on the ratio of the rates of error accumulation in stationary and non-stationary phases. It is almost certain that a stochastic treatment would be needed to obtain significant results. Some predictions, however, seem general. Transformed cultures, in spite of their so-called immortality, should generate slowly dividing or non-dividing cells. If errors of protein synthesis are relevant, transformed lines should "revert" in the presence of amino acid analogues.

If errors accumulate in somatic cells and finally lead to the cessation of cell division, why does the same process fail to occur in the germ line? The simplest escape from this dilemma is to suppose that protein synthesis is sufficiently accurate in germ-line cells to maintain a constant, low error level. In somatic cells the rate may be higher, or may become higher with age, so that an error catastrophe ultimately develops.

It is also possible that "quality control" processes operate during oogenesis and early development, and lead to the rejection of ova or embryos in which the error level is too high. It is known that, in humans, many of the oocytes that begin to mature later degenerate. This could be the result of cellular selection for "metabolic efficiency".

Future Developments

Techniques are now available that permit the measurement of mutation rates and error frequencies in fibroblast and other cell cultures. We can expect to know fairly soon whether or not these quantities vary with the age of the cultures in the manner predicted by error theories. It is important that information of this kind be accumulated for as wide a range as possible of cell types and animal species.

Such information, while valuable, will not be sufficient to enable us to decide whether or not errors of molecular synthesis are among the primary causes of clonal ageing, or whether they are triggered by some different molecular process. Studies of the division times within individual clones of cells may help to resolve this issue. Protein-error theory, for example, would lead one to expect a positive correlation between the division times of mother and daughter cells, even in young or transformed cultures—molecular clock theories do not require such a correlation.

It is still difficult to measure the fidelity of macromolecular synthesis in the tissues of whole animals, but information of this kind should become available soon. Even when we have this information, the difficulty of determining the relevance of such experiments to ageing will be formidable. A low level of errors in any particular type of cell would not rule out the possibility that ageing is due to error accumulation in some other type of cell. Conversely, if the error level is high in some or all tissues of old animals, this could be the consequence of some earlier triggering event, and might have little or no importance for the pathology of ageing.

A connexion between the ageing of cells and the ageing of animals would be suggested if it could be shown that a process that increases the life span of cells in culture produces a corresponding increase in the longevity of the donor species. The demonstration that genetic diseases characterized by premature ageing are associated with inaccurate DNA, RNA or protein synthesis would also count as evidence in favour of a contribution of errors of synthesis to whole-animal ageing.

I believe that it will be a considerable time before the importance of intracellular errors of macromolecular synthesis for *in vivo* ageing can be assessed. It seems clear that immunological mechanisms and the deterioration of extracellular material play an important part in the ageing of mammals. It may be difficult to determine whether these processes are independent of errors of macromolecular synthesis, coupled to them, or a consequence of them.

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Muscular Contraction and Cell Motility

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It has become apparent during the past few years that close similarities exist in a number of instances between some of the proteins directly involved in contraction in striated muscle, and proteins present in certain non-muscle cells in which movement occurs. The question therefore arises whether all these systems share a common basic mechanism, and if so, whether the current picture of the contracting mechanism in striated muscle, which is a fairly detailed one in some respects, can cast any light on these other motile mechanisms, which are less well understood.

Here I first describe certain aspects of the muscle mechanism, especially structural ones, which seem to me particularly relevant to more general questions of motility. Next, I review a number of recent studies which demonstrate in a very decisive way that the similarities between the proteins concerned are ones that relate to the most basic properties and interactions used in the muscle mechanism. Finally, I point out that these considerations suggest a definite mechanism for certain kinds of cell motility. This general type of mechanism ("active shearing") has been suggested by others before[1-3] (though not always in very explicit terms) but it does not seem to have gained general acceptance.

According to the sliding filament model of muscle contraction striated muscles consist of overlapping arrays of actin and myosin filaments which can slide past each other when the muscle changes length. The individual filaments, and the arrays which they form because of their in-register arrangement, remain virtually constant in length. The active sliding force between the filaments is developed by cross-bridges on the thick myosin-containing filaments. These represent the biologically active ends of individual myosin molecules, which can attach to, and exert a longitudinal force on, the thin actin-containing filaments alongside. A cross-bridge is believed to act in a cyclical manner, pulling an actin filament along towards the centre of an A-band for a distance which is probably of the order of 50 to 100 Å, then releasing and reattaching to the actin filament at another point, initially further away from the centre of the A-band and going through the cycle again. A continuous movement of the actin filament is thus produced by the asynchronous action of all the cross-bridges acting upon it from the myosin filaments alongside.

The mechanism requires that the molecules of myosin and actin are assembled in their respective filaments with the appropriate structural polarity. As the cross-bridges have to move so as to draw the actin filaments towards the centre of the A-band, they must be oriented so that they pull in one direction in one half A-band and in the opposite direction in the other half. Thus all the myosin molecules must be oriented in one sense in one half of the length of each thick filament, and in the opposite sense in the other half. This is indeed found to be the case in practice[4]. Also myosin molecules are able to assemble *in vitro* into filaments with this important and characteristic reversal of polarity half way along their length. A similar requirement applies to the actin filaments. Highly specific interactions between an actin monomer and a myosin cross-bridge require that the interacting groups on the two molecules always have the appropriate mutual orientation. Thus actin filaments which interact with opposite ends of a myosin filament must contain actin monomers with opposite polarity. In practice, it is found that all the actin monomers along a given actin filament have the same polarity, and that the polarity reverses at the Z-lines. Thus the actin filaments are attached on either side of the Z-lines with opposite polarity[4].

This means that the direction of the relative force experienced by an actin filament when it interacts with appropriately oriented myosin filaments is specified by the structural polarity of the actin filament itself. Consequently, if an actin filament were attached to some other cellular structure with the same polarity as actin filaments are attached to Z-lines, then the attachment point would experience a force pulling it in the direction of the actin filament. Again, as the force exerted on an actin filament must always be in the same direction, such a filament (or group of similarity polarized filaments) might be maintained in motion over significant distances by interaction with myosin filaments[4], whose orientation could be selected and perhaps enforced by the actin. These considerations are obviously relevant to mechanisms for cell motility and will be discussed again later.

A second characteristic of the sliding filament mechanism as it appears to work in practice is that the relative force between myosin and actin is developed as the result either of an active change in the angle of attachment of the head of the myosin molecules (the S_1 subunit) to the actin filament (Fig. 1), or of an active change in the shape of the S_1

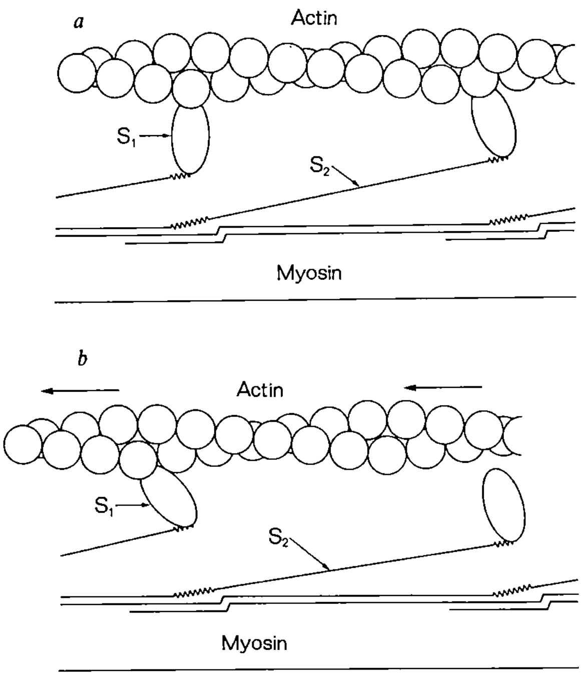

Fig. 1 Active change in angle of attachment of cross-bridges (S_1 subunits) to actin filaments could produce relative sliding movement between filaments maintained at constant lateral separation (for small changes in muscle length) by long range force balance. Bridges can act asynchronously as subunit and helical periodicities differ in the actin and myosin filaments. Only one of the two S_1 subunits in each myosin molecule is shown. *a*, Left hand bridge has just attached; other bridge is already partly tilted. *b*, Left hand bridge has just come to the end of its working stroke; other bridge has already detached, and will probably not be able to attach to this actin filament again until further sliding brings helically arranged sites on actin into favourable orientation.

subunit, the attachment to actin remaining rigidly fixed[5]. That is, the relative sliding force appears not to be generated by changes elsewhere in the myosin molecule or filament, not, for example, by an active change in the orientation of the S_1 head generated at the link to the S_2 part of the molecule. The arguments supporting this view, which I believe are very powerful are somewhat involved, and the original papers should be consulted[4,6,7]. In essence, they derive mainly from X-ray diffraction and electron microscope evidence that the attachment of the heads of the myosin molecules to the backbone of the thick filament is very flexible, whereas their attachment to the actin filaments (in the rigor configuration) is a very rigid one.

If this picture is correct, it follows that the basic contractile mechanism is represented by the interacting complex of thin filament and attached myosin head, and that the exact mode of assembly of myosin molecules into filaments, provided their appropriate polarity is maintained, may be capable of some variation. Even amongst striated muscles, variations are observed in non-vertebrate species, and it is perfectly possible that myosin may be organized in quite different forms in other situations whilst the same myosin head-actin filament interaction is still maintained.

A third feature of the sliding filament mechanism in striated muscle is concerned with the exact mode of attachment of the myosin head to the actin filaments. At present, no means are available to arrest the working stroke of the cross-bridge, or to prevent subsequent dissociation in the presence of ATP. In the absence of ATP, however, the myosin cross-bridges remain attached to the actin filament and the muscle is then in rigor. In an *in vitro* system, actin filaments can be "decorated" in the absence of ATP, by the attachment of molecules of myosin or of heavy meromyosin (HMM) or of subfragment 1 (S_1). Suggestion of an "angled" configuration of the attached myosin heads was given by the visual appearance of "arrowheads" on decorated thin filaments, but the first good evidence was provided by electron microscope studies of thin sections of glycerinated insect flight muscle, and of the X-ray diagrams given by it in rigor and in the relaxed state[8]. More detailed studies of the negatively stained decorated filaments by the three-dimensional image reconstruction technique[9] have shown that not only are the myosin S_1 subunits (which appear as somewhat elongated structure of dimensions very approximately 150 Å×40 Å×30 Å) tilted at an angle of about 45° to the long axis of the actin filaments, but they are slewed round in a characteristic way, that is rotated by about 45° in a plane parallel to the axis of the actin filament and perpendicular to the plane in which they are tilting. It is this combination of tilting and slewing, together with the distinctive shape of the myosin heads, which gives rise to the very characteristic "arrowhead" appearance (Fig. 2), which would not arise from a straightforward "tilted" attachment. We do not at present know the functional significance of this peculiar feature of the attachment (the tilt itself is, of course, what we should expect to find at the end of the working stroke if the initial attachment was perpendicular, and is in the right direction); but it is diagnostic of a very specific structural interaction and of a very specific shape of the attached molecule. It has been found in many cases, as I will describe, that proteins which may be involved in non-muscular motility can form complexes of virtually identical structure.

A final feature of the contraction mechanism in striated muscle is concerned with regulation of activity, that is with switching contractile activity on and off. This is effected by preventing the attachment of the myosin heads to actin in a relaxed muscle, and allowing it to take place when the muscle is activated. Attachment to actin is a necessary part of the biochemical cycle in which ATP is split and force developed by the actin-myosin system. In its absence, ATP splitting, by the myosin alone, takes place very slowly and no tension is developed by the sliding filament system. Indeed, the filaments will slide past each other passively under the action of relatively small external forces.

In a relaxed muscle, the concentration of free calcium is kept at a very low value (probably less than 10^{-7} to 10^{-8} M) by the action of the calcium pump in the sarcoplasmic reticulum. Upon activation, calcium is released so that the level of free calcium rises (probably to 10^{-6} to 10^{-5} M) and attachment of myosin to actin can take place. In vertebrate striated muscle, this change is effected by the tropomyosin-troponin system in the actin-containing filaments[10], which prevents attachment of myosin in the absence of free calcium, but allows it to take place in its presence, possibly by a steric blocking mechanism dependent on changes in position of tropomyosin. In molluscan muscles, however (including the striated muscle of *Pecten*), regulation is effected by changes in the myosin molecules to which calcium ions become bound upon activation[11]. The distribution of these two types of regulation, or combinations of them, between species is a subject of very active current research[12]. Nevertheless, all systems so far investigated share the common feature that they are operated by changes in the concentration of free calcium ions over the critical range of 10^{-7} to 10^{-5} M.

If one is primarily interested in the molecular mechanism of contraction, then striated muscle is the best system to work with, because of its high degree of order, because of the high concentration and relatively large amounts of the contractile proteins in it, and because of its great robustness and stability, which allow it to be manipulated with ease and fixed for electron microscope examination in a state close to its native one by relatively crude techniques. We must now consider, however, whether the same basic mechanism appears anywhere else in nature,

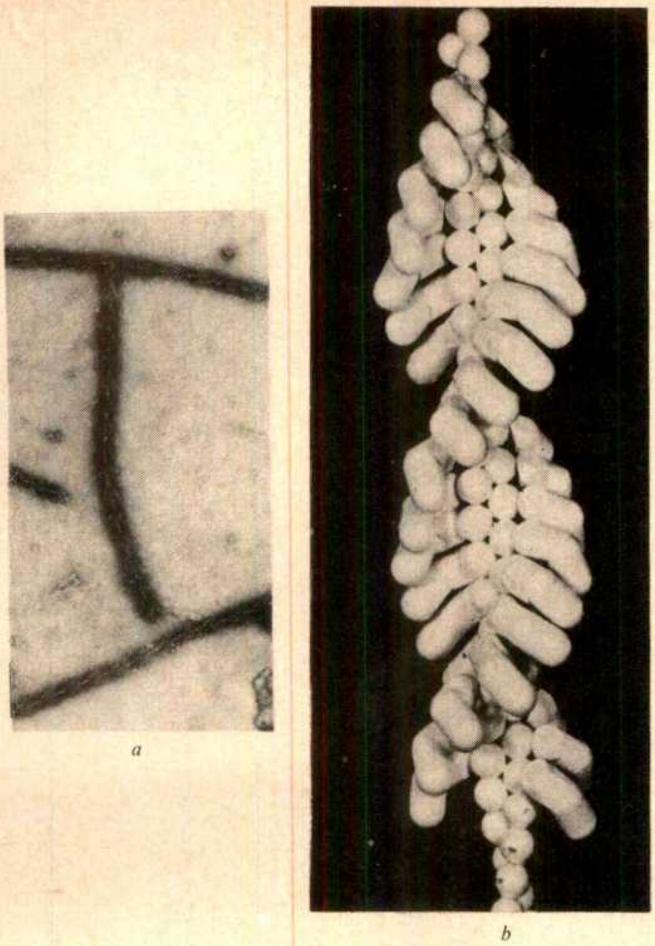

Fig. 2 *a*, Electron micrograph of negatively stained preparation of actin filaments "decorated" with myosin subfragment 1, showing well developed "arrowhead" formations (×138,600). *b*, Simplified model of "decorated" actin filament, based on 3-D reconstruction results; the S_1 subunits are attached to the central core of helically arranged actin subunits in a characteristically tilted and slewed configuration, and it is this that leads to the appearance of arrowheads.

even, in the first instance, in as closely related a system as smooth muscle.

There is no difficulty here with the kind of smooth muscles found, for example, in the adductor and retractors of bivalves; these have been shown to contain abundant thick and thin filaments[13], and the reason that striations are absent is the simple one, suggested by Jean Hanson and myself[14], that the filaments are not arranged in register. It is vertebrate smooth muscle which has until recently presented much more difficulty, as for many years only thin filaments could be seen by electron microscopy and only actin reflexions picked up by X-ray diffraction, in spite of the fact that both actin and myosin were obviously present from biochemical observations.

In the past few years, however, several groups of workers have shown that the form of the myosin component in vertebrate smooth muscles is very dependent on the physical and chemical environment of the muscle; and Lowy *et al.*[15,16] have found that with appropriate conditions before and during fixation, prominent and plentiful ribbon shaped structures can be seen amongst the actin filaments. They have also shown that there is good X-ray evidence for this form of myosin aggregate in comparable specimens before fixation. Some workers believe that cylindrical filaments represent the more natural form of the myosin assembly in smooth muscle, but I think everyone now agrees that myosin is present in smooth muscle in the form of large aggregates and that this probably represents its form when involved in contraction.

The actin component and the regulatory protein system appear to be virtually the same as in vertebrate striated muscle, and the main difference detectable in the purified proteins from smooth muscle lies in the solubility properties of the myosin, that is, its inability to form aggregates with itself at physiological ionic strength except in special conditions. We do not know the functional significance of this, but it is undoubtedly responsible for the difficulties which have occurred in detecting the myosin component by X-ray diffraction and electron microscopy. However, I do not think that there are any serious doubts now that contraction takes place in smooth muscle by the same basic molecular mechanism as in striated muscle and by an essentially similar sliding filament process, although the overall structural organization may be somewhat different.

Non-muscular Systems

Before I consider how some of the other motile systems might function mechanically, it will be helpful to review briefly some of the more recent findings on their protein components. This account cannot do justice to many of the pioneers, but very often technical advances have now made it possible to characterize these proteins much more fully and relate them to the proteins from muscle in a decisive way; and this latter evidence is easier to summarize in a short article.

First, consider the ameoba *Acanthamoeba castellanii*, which has the capacity for typical amoeboid movement. Weihing and Korn[17-19] have extracted an actin-like protein from this organism, which is virtually identical to muscle actin in every respect that can be investigated. It forms the same double helical filaments when examined by negative staining in the electron microscope, and these give exactly the same arrowhead structures when combined with muscle myosin, showing that the myosin-binding sites are oriented in a closely similar way[20]. The amoeba actin can activate the ATPase activity of muscle myosin, possibly to a lesser extent than muscle actin can, and this can be regulated by the tropomyosin-troponin system of rabbit muscle[21]. When the molecule is cleaved by cyanogen bromide, three large peptides can be isolated which are almost identical in amino acid composition to the three corresponding cyanogen bromide peptides from muscle actin[19]. As in muscle actin, one of these peptides contains the rare amino acid 3-methylhistidine. Additionally, amoeba actin contains one residue of ϵ-N-dimethyllysine and small amounts of ϵ-n-monomethyllysine.

Next consider the slime mould *Physarum polycephalum*, which shows very active cytoplasmic streaming. It has been shown by Hatano *et al.*[22-24] that an actin-like protein can be extracted from this organism, which shows the same double helical structure as muscle actin in the electron microscope. As in muscle actin, the monomeric form has one mol of bound ATP which is dephosphorylated to ADP when the actin polymerizes in the presence of salts. Adelman and Taylor[25] have shown that slime mould actin can activate the ATPase activity of muscle myosin, and Nachmias *et al.*[26] have shown that the actin can combine with rabbit muscle myosin subfragment 1 to give arrowheads very closely similar to those characteristic of muscle actin. Again, this shows a very specific structural relationship in the complex.

Next consider blood platelets, which undergo a kind of contraction during clot retraction. Several groups of workers[27-30] have shown that the protein known as thrombosthenin A is closely similar to muscle actin in its structure, in its ability to form arrowhead complexes with muscle myosin (usually HMM), in its ability to activate muscle myosin ATPase, in its ability to allow its activating activity to be regulated by the tropomyosin-troponin system, and in its content of 3-methylhistidine.

Again, consider nerve cells actively growing and extending in tissue culture. Fine and Bray[31] have shown that no less than 20% of all the "soluble" protein in developing chick neurones behaves like actin on an SDS gel. This protein forms the characteristic arrowheads with HMM and shows remarkable chemical similarity to muscle actin on two-dimensional electrophoresis diagrams; of fourteen methionine labelled peptides, ten coincided with ones from muscle actin, showing close homology though not necessarily complete identity.

Further examples could be given, but I think the cases mentioned are sufficient to show decisively that in a number of widely different cells exhibiting different forms of motile activity, a protein closely similar to muscle actin by rather strict criteria is present in significant amounts. And in many cases, the protein can be identified in the cell *in situ*, as bundles of filaments of the appropriate diameter, which can be "decorated" by HMM, and which are in a position where they might be associated with movement[32–34].

In addition to non-muscle actins, myosin-like proteins have also been found in many of these same cells possessing motile activity, and in several instances the protein in question has been identified by rather strict criteria.

In the slime mould *Physarum polycephalum*, Hatano *et al.*[35–37] have isolated a protein which has similar ATPase and actin-combining activities to muscle myosin, the same very characteristic form (being a long rod-shaped molecule with a globular region at one end), and a similar sedimentation constant to muscle myosin (~6.05). Interestingly, the protein differs from striated muscle myosin in being soluble at physiological ionic strength. Adelman and Taylor[25] showed that its molecular weight was close to that of muscle myosin, about 460,000. Nachmias[38,39] showed that the slime mould myosin would attach in the characteristic arrowhead configuration both to slime mould actin and to muscle actin, and also[40] that in the presence of millimolar concentrations of calcium the myosin would assemble itself into rather short but essentially similar bipolar filamentous aggregates like those of muscle myosin.

In the case of blood platelets, Bettex-Gallard *et al.*[41] and Booyse *et al.*[42] have shown that the other component of thrombosthenin is a myosin-like protein, having calcium and magnesium-activated ATPase activity and a molecular weight of approximately 540,000. Adelstein *et al.*[28] have shown that this molecule contains large polypeptide chains of chain weight 200,000, just like muscle myosin, and chains of 16 to 18,000 molecular weight, analogous to the light chains of muscle myosin. The platelet myosin will form excellent arrowhead structures with actin, and will also assemble itself into the characteristic bipolar aggregates at low ionic strength.

In the case of the amoeba *Acanthamoeba*, however, Pollard and Korn[43] have found that, although a myosin-like component can be identified in the sense of having ATPase activity and actin-binding ability, this protein is rather different from muscle myosin in other respects, having a much lower molecular weight (~200,000) and lacking the ability to form filaments. This particular species of amoeba may, of course, be a special case, and it should be remembered that myosin-like filaments have been seen in other amoeba (for example, in *Chaos chaos*[44,45] and in *Proteus*[46,47]).

Thus there are a number of instances where it has been demonstrated quite decisively that cells showing various forms of motility contain proteins which behave in many crucial respects exactly like actin and myosin from muscle. The resemblance is particularly significant in those properties concerned with the activating effect of actin on the ATPase activity of the myosin and with the precise structural form of the actin-myosin complex, as these are so directly linked to the contraction mechanism in striated muscle. It is difficult to believe that such close homology would exist unless the same basic mechanism was involved in each case.

Indeed, one can take this argument a stage further and suggest that the actin filament-myosin head interaction was developed as a motile mechanism very early in evolution, and that cells have been using it ever since, with a high degree of conservation of the essential protein interactions involved. As multicellular organisms developed, certain cells specialized in producing more extensive and more powerful movements, and the contractile proteins in them became organized into the larger structures required to integrate the smaller scale motile processes into directed forces. But the same basic molecular mechanism was retained.

In striated muscle, it has been shown that the mechanism depends on the development of a relative shearing force between filaments of actin and myosin. Because so many of the underlying structural and biochemical features of this interaction are shared by the more primitive systems, it seems highly probable, to say the least, that these systems must also operate by an active shearing mechanism, in which sliding forces are developed between polarized actin filaments and some form of myosin assembly. For example, in cases where cytoplasmic streaming is taking place next to a stationary cortical gel layer, one might suppose that actin filaments, attached to the inner cell surface and lying approximately parallel to it, with appropriate structural polarity, generate an active shearing force by their interaction with assemblies of myosin molecules in the more fluid cytoplasm, which is therefore propelled along, carrying with it other cell organelles. If the myosin assemblies, which could be quite small, contained two sets of myosin molecules with opposite polarity (either in the form of bipolar filaments, or perhaps as "face-polar" sheets as described in smooth muscle by Lowy *et al.*[48] and Small and Squire[49]), then the second set could interact with actin filaments of appropriate polarity in solution, and propel those along too. Alternatively, the location of the actin and myosin assemblies could be interchanged.

Cell organelles could be moved more directly by means of attached actin filaments, which could interact with myosin in large or even very small assemblies and propel themselves along in a manner which I suggested some years ago[4], or by the interactions described above.

Where a cell, for instance in tissue culture, is moving over a substratum, it is clear that one part must be anchored to the support and that another part of the cell must move relative to that attachment site and form new attachment sites of its own. As attachment sites on and between cells often show filaments trailing back from them, it is natural to suggest that those filaments represent one component of an active shearing system, say, actin, and that the other components are in the cytoplasm and therefore can flow forward over the attached filaments. The filament attachment sites, perhaps analogous to fragments of Z-lines, would pass through the membrane and anchor to the substratum. As the front part of the cell was pushed forward by the internal pressure generated by the cytoplasmic stream, fresh attachment sites for actin filaments might be laid down at the leading edge. As actin filaments polymerized onto these (with appropriate polarity) they would be pulled back by shearing forces developed with the cytoplasm. This might give rise to the appearance of "ruffling" and backwards flow of the unattached membrane of moving cells. When the attachment sites became anchored to the substratum, however, the cell as a whole could then move forward as before over the attachment point (Fig. 3). (See also Bray, *Nature New Biology*, in the press.)

The character of encounters with other cells would depend on whether the external attachment sites on the two interacting cells could attach to each other, or only

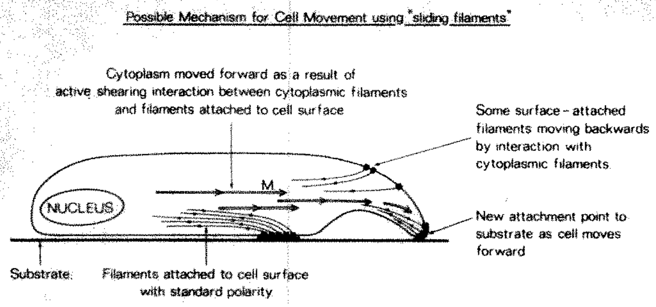
Possible Mechanism for Cell Movement using "sliding filaments"
Cytoplasm moved forward as a result of active shearing interaction between cytoplasmic filaments and filaments attached to cell surface
Some surface-attached filaments moving backwards by interaction with cytoplasmic filaments
M
NUCLEUS
New attachment point to substrate as cell moves forward
Substrate
Filaments attached to cell surface with standard polarity

Fig. 3 Diagrammatic representation of a mechanism by which active shearing forces developed between two sets of filaments could produce cytoplasmic streaming and cell movement.

to a third, freely movable site on the opposing membrane. In the latter case, the attachment sites between cells would be drawn to their edge, sustained overlap could not occur, and indeed the entire actin complement of that region of the cell might become tied up in such junctions, leading to "contact inhibition"[50]. In the former, then, overgrowth could occur. Whilst these latter suggestions are entirely speculative, the force of the earlier arguments may make them worth considering and testing.

Another problem, about which there is very little experimental evidence at present, concerns the regulation mechanisms used in non-muscular motile systems. Because activity in muscle is controlled by small changes in calcium concentration, it would be reasonable to look for similar changes, and for the necessary calcium sequestering structures, in other motile systems. Evidence for the presence of elements of the troponin-tropomyosin system in slime mould[51] and in platelets[52] has recently been described, and evidence for the presence of calcium-sequestering structures has been described in *Spirostomum*[53] and *Physarum*[54].

Finally, I should mention that there are now numerous other instances where actin-like filaments have been implicated in cell movement, though the evidence does not have the decisive character of the examples I have given earlier. In many of these, particularly in the cases of various types of morphogenetic movement, biochemical identification of actin will be difficult. There are, however, several instances where actin has been identified by arrowhead formation with HMM[55]. This is a very specific test for an actin-like protein (though not necessarily for its location in polymerized form unless actin-like filaments can be seen in the same position in untreated material). For example, Perry *et al.*[56] showed that a ring of filaments, just below the surface of the cleavage furrow at the newt egg, would bind HMM to give arrowhead complexes. This ring of filaments has been described by several workers on jellyfish eggs[57,58], and also on the eggs of a polychaete worm[58] and on the ciliate *Nassula*[59] as a contractile structure which, by decreasing its diameter, is responsible for at least a large part of the cleavage process. Two opposing sets of actin filament, linked by myosin, could have this property.

Tilney and Mooseker[60] have shown that filaments located in the microvilli forming the brush border of epithelial cells of chicken intestine also give arrowheads with HMM, behave like actin on SDS gels and, especially interestingly, appear clearly to be anchored to the cell membrane; thus they may aid transport by some kind of pumping action.

In summary then, there seems to be good evidence that several types of cell movement are brought about by molecular mechanisms which use proteins very similar to actin and myosin in muscle. A strong presumption exists, therefore, that active sliding or shearing mechanisms are involved. This possibility needs to be explored in each case, especially by structural and mechanical studies on the detailed processes involved.

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Transformation of Mammalian Cells *in vitro* by Low Doses of X-rays

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X-irradiation of hamster embryo cells in culture, followed by cloning of the cells, was used to obtain a dose–response curve in terms of cell transformation. Transformations were observed for doses as low as 1 rad.

PREVIOUS reports have described the induction of malignant cell transformation by a dose of 300 rad of X-rays in short term cultures of hamster embryonic cells[1-4]. The conditions required for the fixation of this transforming event as a hereditary property of the cells have also been reported[1,3,4], as well as some of the *in vitro* characteristics of the X-ray transformed cells[1-6] and their capacity to induce tumours *in vivo*[1,2]. Recently, some of these investigations have been repeated using established mouse cell lines[7]. A comprehensive study of the carcinogenic action of radiation must include a knowledge, at the cellular level, of the relationship between the absorbed dose and the probability of neoplastic conversion.

This report describes experiments designed to elucidate the dose–response relationship over a range of doses from 600 rad down to 1 rad.

Experimental Techniques

Minced midterm whole embryos from golden hamsters were used as the source of normal cells. Primary cultures were established by progressive dissociation of the minced fresh tissue[8]. Cells (10^7) were seeded into each 100-mm Petri dish (Falcon Co.) and incubated at 37° C in a humidified incubator with 5% CO_2 in air. Three-day-old primary cultures were prepared into a cell suspension by trypsinization, and cloned into 60-mm Petri dishes on X-irradiated (4,000 rad) feeder cells of the same type[9]. Seeding levels ranged from two thousand to ten thousand cells, depending on the dose to be administered, thus allowing for cell killing.

In the experiments to study transformations, the cells were irradiated at room temperature, 24 h after seeding, with a range of doses from 1 to 600 rad. The source of X-rays was a 210 kV Westinghouse constant potential generator, with added filters of 0.5 mm of copper and 1 mm of aluminium. For the higher doses (75–600 rad) a treatment distance of 50 cm was used, corresponding to a dose-rate of 70.6 rad min^{-1}. For the lower doses (1–10 rad) a longer treatment distance of 250 cm was used at which the dose-rate was 4.25 rad min^{-1}; the lower dose-rate was used to avoid shutter errors associated with very short exposure times. Doses were measured with a Victoreen R-meter, calibrated at the Bureau of Standards.

After a post-irradiation incubation period of 9 d the clones were fixed and stained[1]. A differential count was made of normal and transformed clones, the latter being identified by piled-up morphology, random cell orientation and loss of contact inhibition, characteristics not seen in the untreated control cultures[1-4].

In several experiments, transformed clones were isolated and grown into mass cultures. The ability of the cells to form clones in semi-soft agar[10,11] and their agglutinability by concanavalin A and wheat germ agglutinin (provided by M. M.

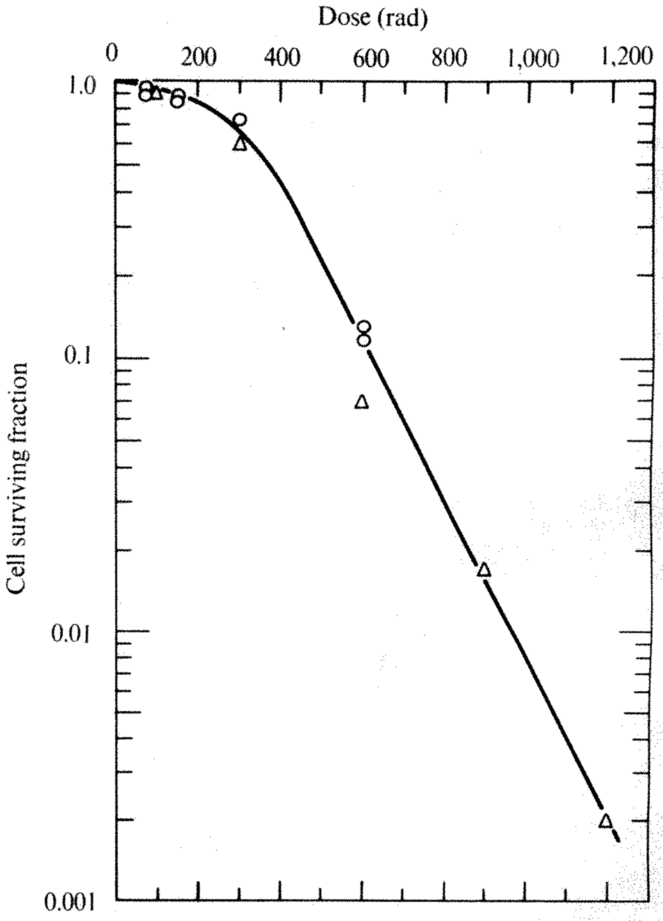

Fig. 1 Survival of reproductive integrity of hamster embryo cells as a function of X-ray dose. Circles describe points calculated from the transformation experiments (Table 1). Triangles describe points established from a separate cell survival experiment (see text). *N*, The extrapolation number, defined as the point in the surviving fraction axis to which the straight portion of the surviving curve extrapolates, =6. D_0, The dose required to reduce the population to a fraction of 37% (used as a measure of the slope in the linear portion of the survival curve), =147 rad.

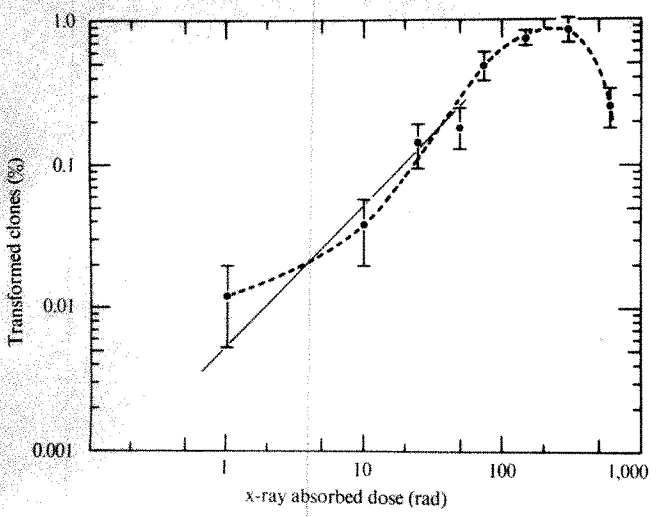

Fig. 2 Incidence of hamster embryo cell transformation following exposure *in vitro* to X-irradiation. For doses at which more than one experiment was performed, the data were pooled; the mean value together with the standard deviation is plotted in the figure (see Table 1 for results of individual experiments). The broken line is drawn by eye to the mean data points; the full line has a slope of +1 and passes through the error bars of each datum point.

Burger)[6,12,13] were used as further criteria for the transformed nature of the cells as compared with control cultures.

Dose–Response Relationship for Transformation

The data for cell survival are shown in Fig. 1, in which dose is plotted on a linear scale against surviving fraction on a logarithmic scale. The shape of the survival curve is characteristic of that commonly observed for mammalian cells exposed to sparsely ionizing radiations, such as X-rays[14]. At low doses there is a broad initial shoulder, implying that X-rays are relatively inefficient at killing cells in this region; for larger doses the survival curve becomes a straight line in this semi-logarithmic plot, implying that over this dose range cell survival is an exponential function of dose.

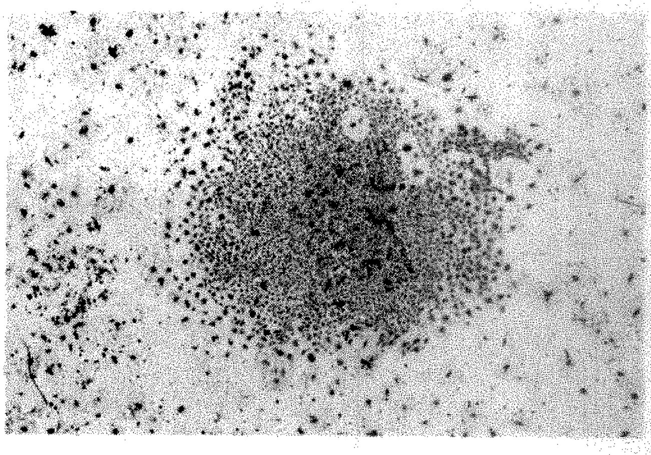

Fig. 3 A 9-d-old clone of normal embryo cells. Giemsa stain ×11.

The dose–response data for transformation are shown in Table 1 and Fig. 2, in which dose is plotted against transformation (%) on a double logarithmic plot. The percentage of cells transformed increases as the dose is increased up to a plateau of approximately 1%, which corresponds to doses of between 150 and 300 rad. A further increase in dose results in a marked decrease in the proportion of cells transformed. Photographs of representative clones, both normal and transformed, are shown in Figs 3–6.

From an examination of the stained cultures it was apparent that the dominant type of transformed clone consisted of cells which were piled up, one on another, having lost contact inhibition[15], but were less randomly orientated than hamster cells transformed by some oncogenic viruses or chemical agents[1]. This morphology has also been observed when tumours induced by X-ray transformed cells were cultured and cloned *in vitro* (C. B., unpublished). Transformed clones of epithelioid morphology[8,13] were found at a very low frequency. In contrast to controls, the X-ray transformed cells derived from all of the ten isolated clones were agglutinable by 50 μg ml^{-1} of concanavalin A or wheat germ agglutinin. The ability to form colonies in 0.33% agar was limited to the cells of the two isolated transformed clones of epithelioid morphology.

Table 1 Neoplastic Cell Transformation of Hamster Embryo Cells following X-Irradiation *in vitro*

	Control			Irradiated				
Experiment No.	No. of clones counted	Plating* efficiency	No. of transformed clones	Dose (rad)	No. of clones counted	No. of transformed clones	Transformed† clones (%)	Surviving‡ fraction
1	1,600	1.6	0	600	920	2	0.22	0.12
2	1,002	2.2	0	600	3,020	8	0.27	0.13
3	1,012	1.3	0	300	1,130	8	0.71	0.75
4	1,220	1.6	0	300	1,960	18	0.92	0.75
5	2,500	4.5	0	150	6,200	44	0.71	0.89
6	4,020	2.1	0	150	3,268	25	0.77	0.86
7	1,620	2.0	0	75	2,132	11	0.52	0.92
8	4,100	1.2	0	75	2,200	10	0.46	0.95
9	1,025	1.05	0	50	5,402	10	0.185	0.95
9	1,025	1.05		25	5,503	8	0.145	1.00
6				10	10,200	4	0.039	1.00
8				1	6,800	1	0.015	1.00
10	9,600	2.5	0	1	9,200	1	0.011	1.00
10	7,200	1.9	0	1	7,900	1	0.013	1.00

* Plating efficiency (P.E.) = $\frac{\text{Number of cells plated}}{\text{Number of clones counted}} \times 100$

† Cell transformation (%) = $\frac{\text{Number of transformed clones}}{\text{Number of clones counted}} \times 100$

‡ Surviving fraction = $\frac{\text{Number of cells plated}}{\text{Number of clones counted} \times \text{P.E.}}$

A preliminary study of the karyotypes of the X-ray transformed cells using the Giemsa banding method[16] indicates that the cells tend to be aneuploid but there are, apparently, no gross chromosomal aberrations. Experiments kindly conducted by Dr I. B. Weinstein, using reported techniques[17], demonstrated that there were no C-type virus particles present in X-ray transformed cells which had been proved to be able to induce tumours[1].

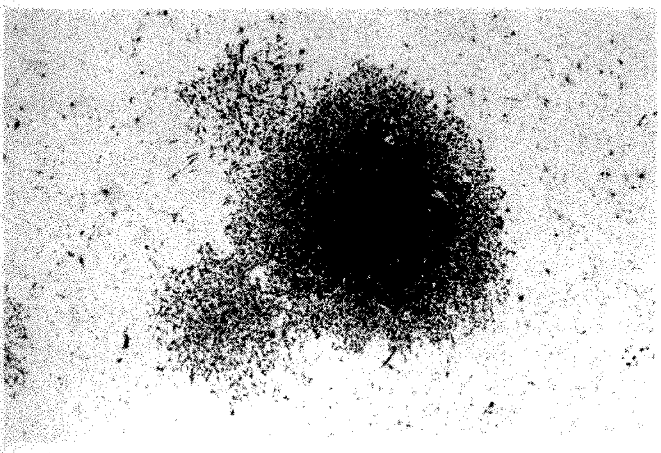

Fig. 4 A 9-d-old clone of hamster embryo cells transformed *in vitro* by 300 rad of X-irradiation. Giemsa × 11.

These results demonstrate that neoplastic transformation can be induced in single cells with doses of X-rays as low as 1 rad. At any of the doses used, the fraction of cells killed is small, and this, coupled with the fact that no transformed clones have been observed in control cultures, strongly supports the view that transformation is directly induced by X-irradiation, and is not a result of a selection of spontaneously occurring transformed cells.

Dose–response relationships for the induction of neoplasms by ionizing radiation have been studied and discussed previously, using human and animal data[18–21]. The shape of the dose–response curve obtained in the present *in vitro* experiments is not unlike that characteristic of *in vivo* data. It consists of three portions; an ascending limb, a plateau and a descending limb.

In the *in vivo* situation there are insuperable difficulties involved in obtaining significant data for low doses of radiation in the range of 1–25 rad, where tumour incidence is low and inordinately large numbers of animals would be required to detect it. Consequently there has been much speculation concerning the actual shape of the ascending limb of the curve. Linear extrapolation from higher doses, on the one hand, has been the conservative approach, while the existence of a threshold dose below which there is no effect cannot be ruled out[22,23]. One of the principal assets of the *in vitro* system is that a very low frequency of transformation can be detected by irradiating large numbers of isolated single cells and scoring thousands of clones. A cell which has received a "hit" at a crucial site and been converted to a neoplastic state can replicate freely and thereby fix this event as a hereditary property[2], and grow into a transformed clone.

If a curve is naively drawn through the data points in Fig. 2, it would have a slope of less than unity for doses between 1 and 10 rad, which on this double logarithmic plot would imply that the transformation rate increases with a power of the dose that is less than one. This kind of dependence has been observed for the induction of mammary neoplasms by neutrons. Rossi and Kellerer[24] have pointed out that in that instance it was necessary to assume a multicellular mechanism of tumour induction because the number of neutron secondaries per cell was considerably less than one. In our experiments the number of X-ray secondaries (electrons) per cell, or even per cell nucleus, is considerably larger than one and the shallow slope which appears to fit the data best may be due to a range of susceptibility among the cells. But the confidence limits on the data points, particularly at the lower doses, are such that one can draw a line with slope +1 that does not fall outside the error bars of any of the points. A linear dependence of induction on dose therefore cannot be ruled out.

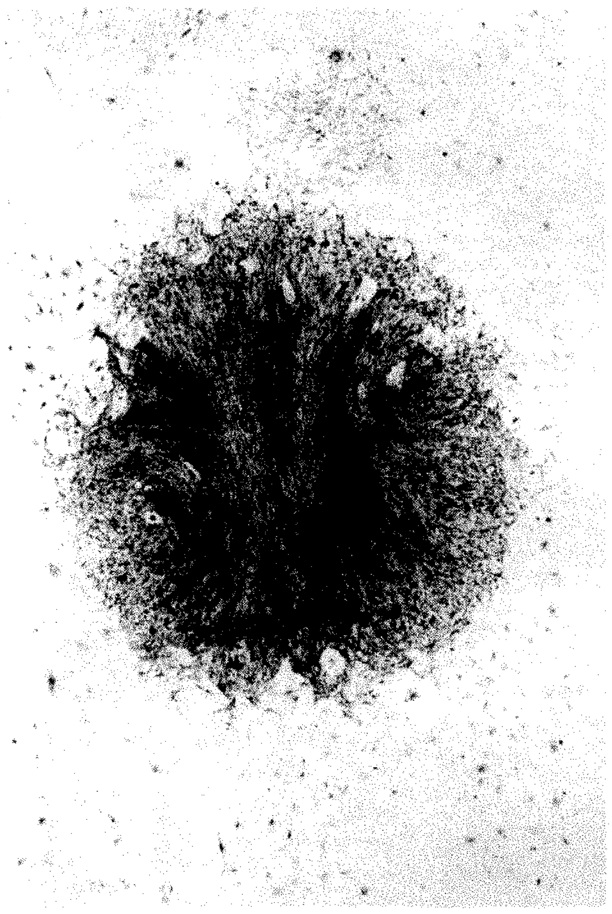

Fig. 5 A 9-d-old clone of hamster embryo cells transformed *in vitro* by 1 rad of X-irradiation. Note the random orientation on the circumference. Giemsa × 15.

The plateau between about 150 and 300 rad closely resembles that often reported for *in vivo* experiments[20]. For a change in dose by a factor of two, the frequency of neoplastic conversion, which is about 1%, remains essentially unaltered. A further increase in dose above 300 rad results in a marked decrease in the frequency of transformation. Similar observations made in humans and animals[18–21] have resulted in speculative interpretations suggesting that the overall high cell killing occurring in these multi-hit dose ranges, and reflected in the survival curves, is responsible for the sharp decline in capacity to induce tumours.

Our data support the idea that above about 300 rad, cell reproductive death[25] begins to be an important factor; the percentage of transformed clones declines above 300 rad in spite of the fact that they are scored from amongst the survivors of the irradiated population, implying that there is a preferential killing of potentially transformed cells. If normal and poten-

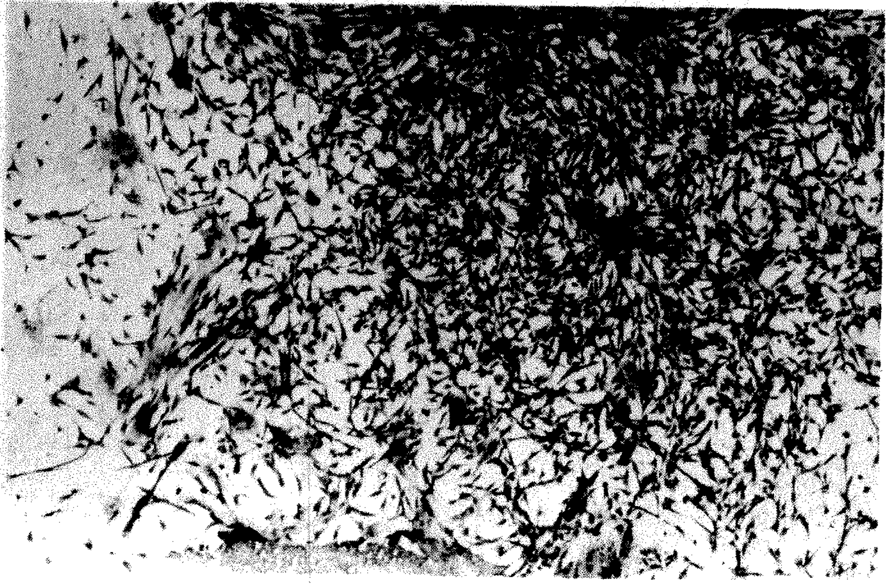

Fig. 6 A section of the circumference of the clone in Fig. 5. Giemsa ×45.

tially transformed cells were equally sensitive to radiation, then a decline in the proportion of transformed cells at higher doses would not be expected. These results support the ideas proposed by Gray[21] inasmuch as they suggest that cells that have been altered by the absorption of radiant energy and are destined to become transformed cells, are more susceptible to being killed by the accumulation of additional damage at higher doses. In other words, many of the potentially transformed cells never have the chance to form a colony because they die a reproductive death due to the extra damage produced by the additional "hits" received at larger doses.

One of the drawbacks of this technique stems from the use of the whole embryo, as a result of which there is a mixture of many cell types. Furthermore, the cell cultures are asynchronous at the time of irradiation, so not all cells are equally at risk. Differences in cell sensitivity to low doses of radiation at various stages of the cell cycle have been reported[25-27].

Our preliminary karyotype analysis of the cells transformed by a dose of 300 rad of X-rays revealed no gross chromosomal aberrations within the limitations of the banding technique used[16]. The event that leads to transformation, however, may well be a subtle genetic structural alteration, such as a gene mutation, which results in the loss of control of cell replication and alteration in the cell surface.

While all the X-ray transformed cells exhibited a microstructural surface change characteristic of the neoplastic state and detectable by plant agglutinins[6,12,13], not all of them form colonies in semisoft agar. As mentioned previously, most of the X-ray transformed cells which are fibroblast-like, and the cells grown from tumours induced by these transformed cells, are not as randomly oriented as those transformed by oncogenic viruses or chemicals. This suggests that these cells have not completely lost "anchorage dependence"[28] which is a requirement for growth in a semi-solid medium such as agar. The cell type which did grow in agar was of epithelioid origin with different growth patterns. They multilayered as rounded cells exhibiting very little adhesion to one another and to the surface on which they were grown.

Some types of leukaemia induced by X-irradiation have been found to be associated with the presence of viruses, implying an indirect action of the ionizing radiation[20,29]. Our findings indicate the absence of C-type virus particles in transformed cells which on injection into newborn hamsters give rise to fibrosarcomas.

In vivo studies on neoplasias induced by X-rays have stressed the wide range of tumour types which arise[18-21]. The fact that neoplastic transformation induced *in vitro* by X-rays results in the appearance of more than one type of transformed cell, together with a striking similarity between the *in vivo* and *in vitro* dose–response relationships, make this system suitable for further studies on the carcinogenic action of X-rays and other ionizing radiations present and increasing in our environment.

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Seafloor Spreading in the Tasman Sea

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Magnetic and seismic studies in the Central Tasman Sea show conclusively that it evolved by a process of seafloor spreading between 60 and 80 m.y. BP, suggesting that other marginal basins may have a similar history. A reconstruction of Australia, the Lord Howe Rise, New Zealand and Antarctica based on these data is presented and discussed.

TECTONIC theory can account for the first-order features of the principal ocean basins, but the origin of the marginal basins of the western Pacific is still in doubt. The crust underlying the marginal basins is similar in thickness and compressional wave velocity characteristics to that of the major ocean basins[1,2], but the depth of the seafloor in these marginal seas is somewhat variable, chiefly because of sediments on the underlying crust. Karig[3] concluded that marginal basins were formed by crustal extensions and that their genesis was related to the proximal arc-trench systems and associated seismic Benioff zones. An alternative proposal calls for injection of mantle material along rifts within or behind an island arc, thus producing a "half-cell" style of seafloor spreading[4].

The Tasman Basin, the West Philippine Basin, and South Fiji Basin were classified by Karig as inactive, marginal basins with normal heat flow[3]. The normal heat flow was taken as evidence that the basins had been devoid of major extensional tectonism for the past 20 to 40 m.y. Karig[3] goes on to say "it would be hazardous to assume the presence of identifiable linear magnetic anomalies in marginal basins until at least one diagnostic survey confirms that assumption".

Here we report new data from the Tasman Sea, which reliably confirm identifiable linear magnetic anomalies which can be recognized as part of the worldwide pattern of Cainozoic spreading lineations[5]. These data, together with seismic reflexion profiling and detailed bathymetric studies, provide conclusive evidence that the Central Tasman Sea has evolved through a process of seafloor spreading similar to that taking place at the seismically active crests of the mid-oceanic ridges. These processes have been dormant within the Central Tasman Basin for about the past 60 m.y. and the area is now aseismic.

The Tasman Sea is bounded on the west by the Australian mainland and its southern extension into Tasmania and the Tasman Rise system; on the east and northeast by the Lord Howe Rise, New Zealand and the Macquarie Ridge Complex (Fig. 1*a*). The southern boundary is poorly defined but is often taken to be the crest of the South-east Indian Ridge System. Magnetic lineations have been mapped previously in the most southern portion of the Tasman Sea and have been related directly to seafloor spreading about the South-east Indian Ridge and to the migration of Australia away from the Antarctic continent commencing about 50 to 60 m.y. ago[6]. Weissel and Hayes[6] and Hayes and Conolly[7] suggested that a morphologic and crustal boundary separated the South Tasman Sea from the Central Tasman Sea along a line extending northeast from the South Tasman Rise to the Resolution Ridge near Fiordland, New Zealand (Fig. 3). We have recognized for some time a major contrast in the sediment disposition of the Tasman seafloor in the area to the north and south of this hypothetical boundary.

Ringis[8] has correlated northwesterly trending magnetic lineations offset along northeast–southwest lines in the Central Tasman Sea, and these lineations were later confirmed by USNS Eltanin[9,10]. Griffiths[11] speculated on the origin of the Tasman Sea by examining the "geological constraints" imposed by the reconstruction of Australia to Antarctica and their interrelation to New Zealand, the Lord Howe Rise and the Campbell Plateau. Griffiths[11] drew on the preliminary data of Ringis[8] to conclude, incorrectly, that the Central Tasman Sea was tectonically active until late Miocene, about 10 m.y. ago. Van der Linden proposed that the Dampier Ridge is a dormant site of seafloor spreading[12], and it has also been suggested that the Tasmantid guyots mark the northward migration of the Tasman Basin across a fixed mantle hot spot, thus recording the motion of Australia away from Antarctica[13]. The inferred age of the Tasman Basin based on the depth of the top of the oceanic crust and simple thermal cooling models was 90±40 m.y. (ref. 13). A cursory examination of widely spaced east–west aeromagnetic profiles between Australia and the Lord Howe Rise led to the early but incorrect conclusion that there were no lineated magnetic anomalies within the Tasman Sea[14].

In Fig. 1*b* we show the tracks along which geophysical data were available to us. Most of these lines were run by USNS Eltanin (47A). The dotted lines represent aeromagnetic flight lines along which magnetic data are the only geophysical parameter available. The other data were collected along ship tracks and most tracks include precision depth recording, seismic reflexion profiling, total intensity magnetic anomaly observations and, in many cases, gravity observations.

This article presents only a small fraction of the new data although our discussion takes cognizance of the much larger total collection of data available for this area.

Magnetic Anomalies

In Fig. 2 selected total intensity magnetic anomaly profiles are aligned on an axis of symmetry and are arranged roughly from north to south, showing not only the correlation of anomaly lineations in this area, but also identifying the individual lineations as a portion of the worldwide Cainozoic pattern[5]. Two of the seven observed profiles shown are aeromagnetic profiles (marked by asterisks) and the somewhat longer apparent wavelengths and attenuated amplitudes are attributed to the increased distance from source to detector compared with anomalies measured at sea level. One synthetic anomaly profile, computed using standard techniques[15,16], is shown near the centre of the figure. Many synthetic profiles were computed (using different natural remanent magnetization parameters) and the profile shown is an example of the range of "optimum" or representative fits for purposes of identifying the observed lineations.

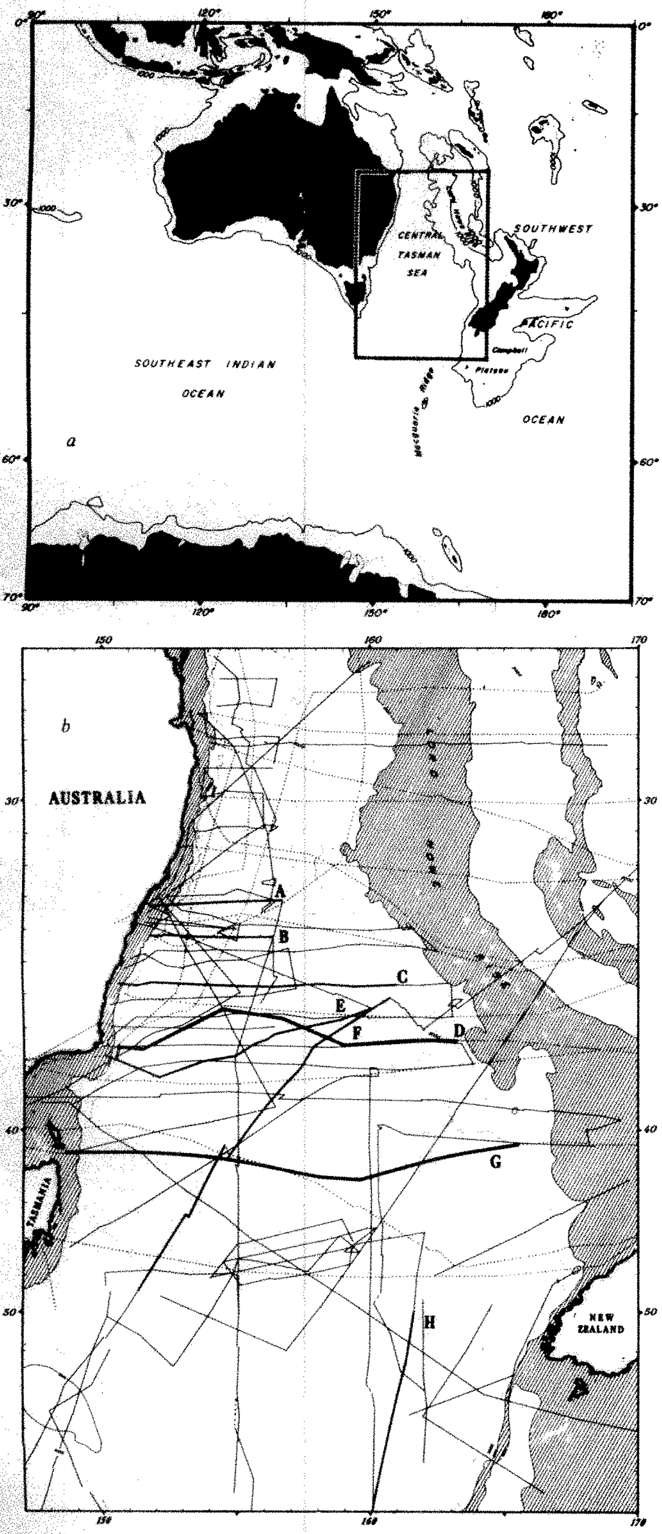

Fig. 1 *a*, Area of study in relation to regional geography. Rectangle encloses area detailed in *b*. *b*, Ship tracks (——) and aeromagnetic flight lines (. . .) along which geophysical data were examined in this study. Letters key selected profiles shown in Figs. 2 and 6.

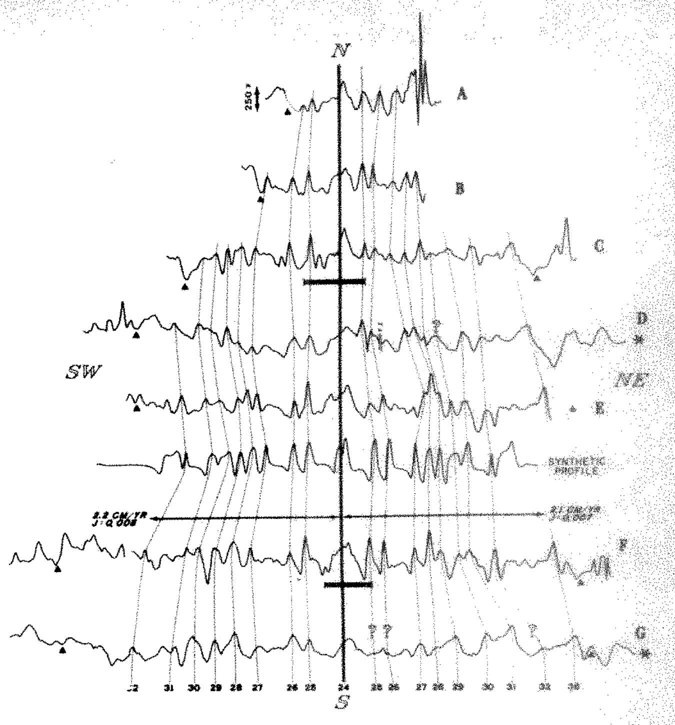

Fig. 2 Selected total intensity magnetic anomaly profiles. Asterisks denote aeromagnetic data. See text for further explanation. Profile locations as Fig. 1*b*.

Although issue may be taken with any single correlation or identification shown in Fig. 2, when one considers the entire pattern and also the many additional profiles that were available, the overall correlations, the lineation ages, and the magnetic fabric are all considered to be very well defined. The inverted carats mark the approximate position of the abrupt increase in slope along the margins of Australia and the Lord Howe Rise. The significance of the slight differences in spreading rates indicated for opposite sides of the synthetic profile will be discussed in a more complete study. The presence or absence of small offsets in the lineation pattern cannot be proven with the reconnaissance data. Such undetected offsets would give rise to small apparent differences in the inferred spreading rates and will introduce complexities in unequivocally identifying all of the anomalies on any particular profile.

The heavy bars shown below profiles C and F mark the position near the crestal zone of the buried basement ridges discussed in the next section. Figure 3 synthesizes the overall magnetic lineations fabric using all the available magnetic, seismic, and topographic data represented by the collective tracks shown in Fig. 1*b*.

Seismic Data

Seismic reflexion profiler data reveal a distinct basement ridge whose axis is coincident with the centre of symmetry of the magnetic lineation pattern previously identified as anomaly 24 (age ~60 m.y. BP). The basement morphology is almost entirely blanketed by a thick cover (>1 s two way reflexion

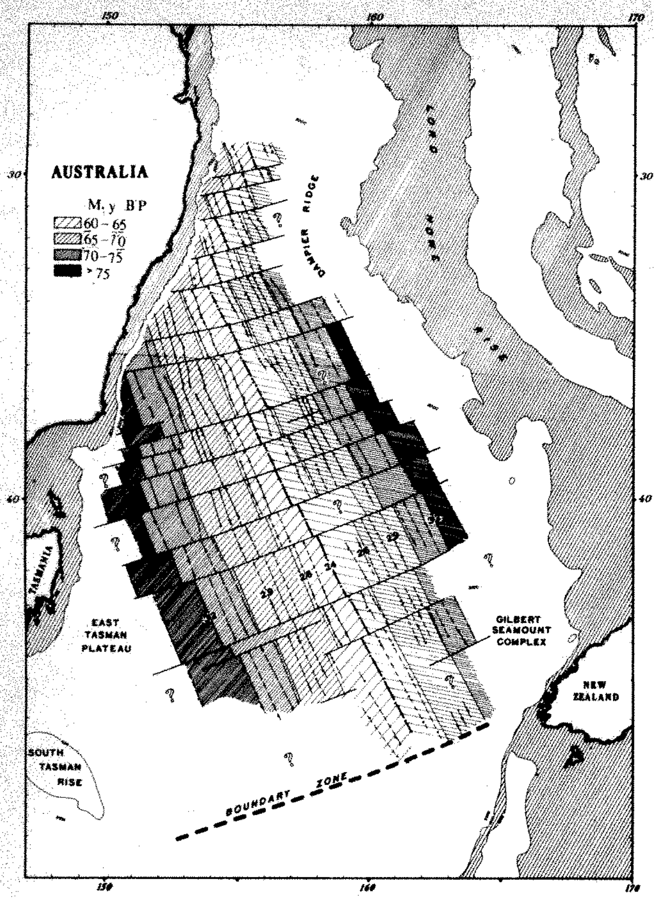

Fig. 3 Magnetic lineations, fracture zones and age of the basement of the Central Tasman Sea. Anomaly identifications (located by dots) and ages according to the Heirtzler *et al.* system[15].

time) of acoustically transparent sediments, which thin subtly near the crest of the buried ridge and in general smooth out both the regional and local relief of the underlying basement surfaces. Some of the larger basement peaks are still expressed (through the semi-conformable sediment layer) in the topography of the seafloor. Only one sub-bottom reflector can be traced over any significant distance, but it is not pronounced, indicating that it represents only a slight change in the physical properties (acoustic impedance) and the inferred sedimentary regime. Adjacent to the Australian continental margin the total sediment thickness is much greater than elsewhere, the primary difference here being the presence of about 0.75 km of layered, flat-lying, presumably interbedded turbidites and hemipelagic sediments. The total thickness of sediments beneath the Tasman abyssal plain was not recorded with our seismic reflexion profiling technique.

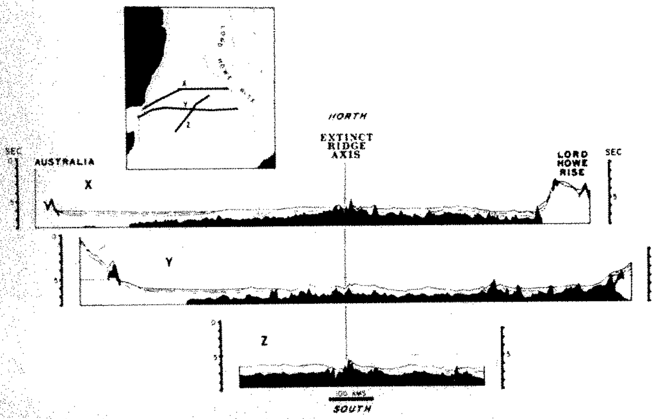

Fig. 4 Line drawings of seismic reflexion profiles. Black areas represent oceanic basement (layer 2; $V_P \approx 5$ km s^{-1}).

The "typical" ridge morphology consisting of a distinct crest, axial rift, and gently sloping symmetric flanks is best developed through the northern half of the Central Tasman Sea and is well illustrated in profile X, Fig. 4, which is nearly orthogonal to the trend of the buried basement ridge. Further to the south, the crest of the ridge is less pronounced (Fig. 4 Y, Z) although still recognizable. Small offset fractures probably transect each of the profiles in Fig. 4.

Using simplified thermal models, a theoretical-empirical relationship has been proposed between the age of the basement crust and its depth below sea level[16] which predicts elevations at the crest of 5,200 ± m and 5,500 ± m at the distant portion of the flanks. The observed depths at the crest range from about 4,500 ± m in the north to about 5,200 ± m in the south. The flanks near Lord Howe Rise are near 5,500 ± m while those near Australia are greater than 6,000 ± m. These results are in fairly good agreement with the models[16] although the elevations of these models are not too sensitive to large age differences for oceanic crust older than about 60 m.y. The greater depth to basement observed along the Australian margin is attributed in part to sediment loading and flexure of the crust, but we cannot exclude the possibility that some limited crustal subduction occurred along this margin while the Central Tasman Sea was actively spreading.

The trend of the buried basement ridge and the magnetic lineation trends are internally consistent, and within the limits of resolution, the axis of symmetry within the magnetic pattern and the axis of the basement ridge are coincident (Fig. 5). The trend of the ridge axis and the magnetic lineation pattern and offsets in the pattern are all oblique to the regional trend of the Victoria–New South Wales portion of the Australian continental margin. The extinct ridge axis and central anomaly (24, ~60 m.y. BP) nearly intersect the Australian continent in the northern portion of the Tasman Sea, just west of the Dampier Ridge (Fig. 3). In addition, the oldest crust recognized in this region is only about 70 m.y. old, or more than 10 m.y. younger than the oldest crust recognized further to the south. Either spreading began somewhat later or was much slower in the northern area than in the southern area, implying a major shear zone between the two, or spreading in both areas was synchronous but the magnetic lineation evidence for the older crust in the north was obliterated by subduction to the west and/or later volcanism on the east. We find no evidence to substantiate the proposal that the Dampier Ridge is an extinct mid-ocean ridge[17]. It seems that the Dampier Ridge is a somewhat younger feature than the adjacent seafloor even though extrapolation of crustal ages to the east of the extinct spreading axis would imply it was an older feature.

A major submarine canyon located just east of Bass Strait coincides with the south-westerly extension of one of the larger offset fracture zones recognized in this area. The offset of the isobaths defining the continental slope are right lateral in sense, consistent with the offset of the ridge axis across the same fracture zone (Fig. 3). This canyon and other similar topographic irregularities along the steep slopes of the Australian margin and western slope of the Lord Howe Rise are probably expressions of fracture zone offsets in the initial pattern of rifting. Similarly, concentrations of Cretaceous and Early Cainozoic volcanism near the Australian coast and the locations of large seamounts are probably controlled by the relict lines of crustal weakness defined by fracture zone offsets in the initial extensional rift.

The southern limit of the late Cretaceous to Palaeocene Tasman Sea opening is now rather clearly defined both on the basis of reflexion profiling data and magnetics data. Figure 6 shows the dramatic contrast in the nature and thickness of the sediment blanket, the depth to the basement layer, and the apparent relief of the basement on opposite sides of the boundary zone. The zone trends east–northeast parallel to

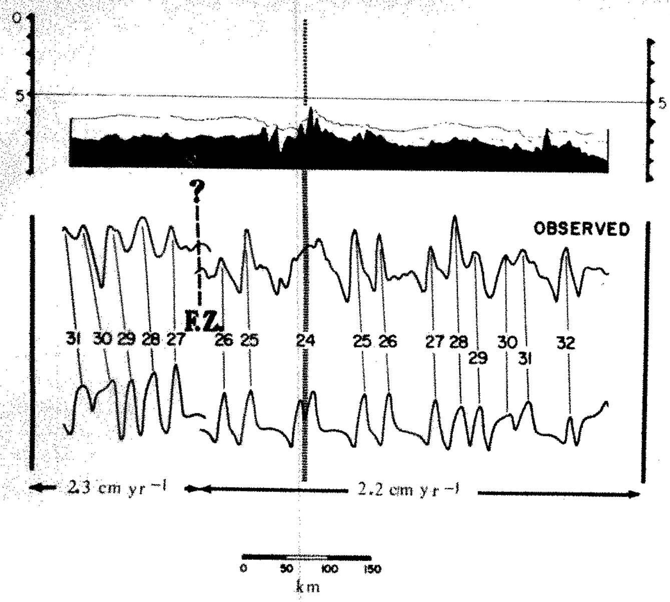

Fig. 5 Profile Z of Fig. 4 together with observed total intensity magnetic anomaly and two dimensional synthetic anomaly, computed using the geomagnetic time scale of Heirtzler *et al.*[15]. We note the coincidence of the axial anomaly (24) and the crest of the buried ridge.

the inferred fracture zones to the north and is expressed as the Resolution Ridge near 46° S, 165° E and extends to the southern limit of the South Tasman Rise near 49.5° S, 152° E. The strike of the boundary zone is also subparallel to the major strike of the Alpine Fault. The tectonic and lineation fabrics north and south of this zone are approximately orthogonal to one another.

This boundary zone must have been the locus of major shearing from 80 to 60 m.y. BP and may have corresponded to the initiation of transcurrent movements along the Alpine Fault or similar fault zones. The orientation and location of the shear zone boundary undoubtedly played a major role in determining the subsequent orientation and locus of the younger rift system (now a part of the Southeast Indian Ridge) along a line of previously established crustal weakness.

Continental Reconstructions

By a trial and error rotation to near coincidence of corresponding magnetic anomaly lineations located on opposite sides of the extinct ridge axis, one can determine a pole and finite rotation angle that best describes the total average relative motion for discrete time intervals[6,18]. If small time intervals are examined in sequence, backward in time, one can approximate the paths of relative motion between the two spreading plates. If, however, one is only concerned with the relative configuration of plates at any given moment of time, a similar procedure may be used but the pole and angle of rotation deduced will not in general define the relative motion for a long time interval[18]. The pole and rotation angle are simply a mathematical convenience in defining the total relative motion and the actual motion could be the resultant of many finite rotations about various poles, but which must sum to the same total rotation about the single pole. Here we simply consider the pre-rifted configuration of the area by determining the pole which brings into closest coincidence the oldest anomaly lineations (36, ~80 m.y. BP, ref. 19) recognized there. Anomaly 36 lies close to the continental edges of east Australia and the Lord Howe Rise as inferred by the positions of the continental slopes. Because of the well developed magnetic pattern, there is not much uncertainty in the pre-rifted configuration of these two continental elements. Using similar techniques the pre-rifted configuration of Australia and Antarctica can be rather precisely determined[6]. The magnetic lineation pattern between the Campbell Plateau and West Antarctica is defined only to the north of the Pacific–Antarctic Ridge; hence the technique cannot be used to reconstruct these two continental elements. An additional complication is introduced by the fact that earthquake epicentres indicate an active plate boundary between the Lord Howe Rise and the Campbell Plateau that is presently defined by the Alpine Fault and the Macquarie Ridge Complex. The nature of this boundary during the late Cretaceous and Cainozoic is in doubt[20–21]. Both of these questions can be investigated by utilizing the constraints imposed by closing up the Central Tasman Sea and the Southeast Indian Ocean as dictated by the magnetic and morphological fabric of these areas.

Figure 7 shows the reconstruction to approximately 80 m.y. BP. The morphological "fits" are far from perfect and significant gaps exist within the incipient Tasman Sea. More important, several large morphological overlaps exist which must be explained if the reconstructions are to be considered valid. The seriousness of the overlaps depends on the issue of the continental versus oceanic nature of the overlapping segments. Continental crust overlapping continental crust such as the case of the Campbell Plateau onto Marie Byrd Land is entirely unacceptable and must be eliminated by hypothesizing additional tectonic motions. In Fig. 7 this particular overlap has been minimized by taking into account the known left-lateral, strike-slip motion across the Alpine Fault of ~480 km which is generally agreed to have occurred in post-Early Cretaceous[21]. The total area of "continental" overlap would be much greater if the Alpine Fault movement had not been reconstituted.

The Dampier Ridge overlaps the area of continental Queensland–New South Wales, Australia. If our reconstruction is correct, it could not have been formed prior to the opening of this area to the extent that the ridge could be accommodated, and we therefore conclude that the most likely mode of formation was by oceanic volcanism subsequent to the initial opening of the Central Tasman Sea, thus accounting for the overlap.

The only other areas of serious overlap are the South Tasman Rise (a presumed extension of the Australia–Tasmania continental block) with the Iselin Plateau (an extension of the Ross shelf, also presumed to be underlain by continental type crust). The continental versus oceanic nature of these features is unconfirmed but will be the subject of two DSDP sites to be drilled during the first two legs of Antarctic drilling[23]. The very shallow depth to high velocity basement rocks at both sites suggests they are partially foundered continental blocks, in which case their overlap is unacceptable.

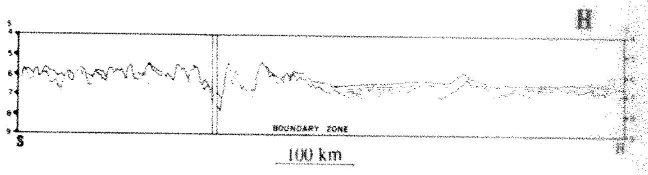

Fig. 6 Line drawing of seismic reflexion profile across the boundary between the Central Tasman Sea and the South Tasman Sea. We note the contrast in sediment disposition. See Fig. 1*b* for profile location.

In order to reconcile the unacceptable overlaps, one must hypothesize plate motions in addition to those in Fig. 7. Because the reconstructions of Australia to Antarctica and of Lord Howe Rise–New Zealand to Australia are considered now to be well determined, we believe the "unknown" plate motions required for an internally consistent picture must either be accounted for by motion between the Lord Howe Rise and the Campbell Plateau or by motion between East Antarctica and West Antarctica.

The total offset of the axis of the New Zealand geosyncline is about 1,200 km with 480 km accounted for by the Alpine

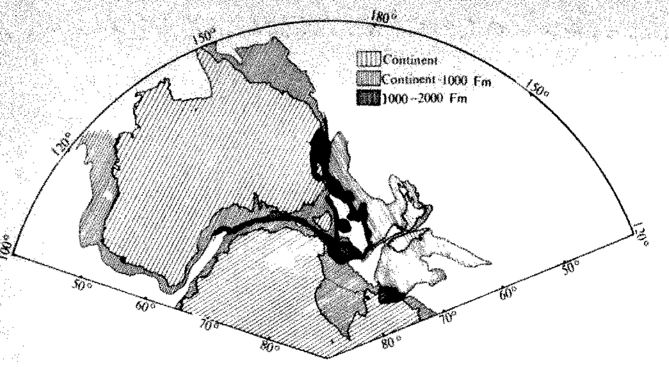

Fig. 7 Reconstruction of Lord Howe Rise, Australia, and Antarctica before about 80 m.y. BP, based on observed magnetic anomaly lineations. The black areas represent regions where there is an overlap onto known continental fragments. See text for detailed discussion.

Fault[11]. Only the Alpine Fault movement has been accounted for in Fig. 7. If the apparent bending and displacement of the geosynclinal axis does in effect represent an additional right lateral offset of about 700 km, this motion would be more than enough to account for the Campbell Plateau to Marie Byrd Land overlap. But this motion would still not reconcile the overlap of the Tasman Rise with the Iselin Plateau. An alternative explanation is a post-Late Cretaceous relative motion between East and West Antarctica. If West Antarctica were to be moved in a right lateral sense to a hypothetical Early Cretaceous position along a line that passes west of the Iselin Plateau, then all of the serious overlaps of Fig. 7 can be reconciled. East and West Antarctica are quite different in their gross geology[24–26], and a major morphological and structural boundary separates the two provinces along a line subparallel to and east of the Transantarctic Mountains. The genesis of this structural zone is entirely speculative although a portion of the area, the Bentley subglacial trench, is characterized by basement rocks that presently lie well below sea level[26].

We favour motion between East and West Antarctica because it allows for the reconciliation of two areas of major overlap and because the motion could be accommodated along the zone of a known structural and geological boundary. The proposed post-Late Cretaceous left lateral motion (~400 km) of East and West Antarctica is entirely speculative. The absence of geophysical data in the eastern Ross Sea precludes an evaluation of our hypothesis for this area, although we would expect to see evidence of limited subduction along the Marie Byrd Land continental margin if our hypothesis is correct.

The best test may come through investigations of the geology and tectonic evolution of other portions of the West Antarctic such as the Antarctic peninsula and adjacent seafloor, where the effects of our proposed plate motions must influence the geology there. It is also possible that the motion of East and West Antarctica we are proposing could be manifested as a rotation and extension[27] rather than pure strike-slip motion. If this were the case, the structural boundary through central Antarctica would represent an area of crustal extension and direct evidence for such an extension might be found by carefully re-examining aeromagnetic data across this area.

Tasman Sea Creation

The Central Tasman Sea was created by spreading about a now buried ridge that was active only from 80 to 60 m.y. BP. The buried axis and the pattern of magnetic lineations are offset along inferred fracture zones that are roughly orthogonal to the ridge and the lineations. Because of their similarity in heat flow, crustal properties and a strong suggestion of lineated magnetic anomalies, it seems likely that some other marginal basins (such as the West Philippine Basin and South Fiji Basin) evolved with histories parallel to that of the Central Tasman Sea.

The Southern Tasman Sea began forming later, ~50 m.y. ago, in direct relationship to Australia's northward migration away from Antarctica through processes of seafloor spreading. The magnetic and morphologic properties of the central and southern regions of the Tasman Sea are nearly orthogonal and the fracture zone fabric of the former may have controlled the orientation of the ridge fabric of the latter.

A distinct basement and morphologic boundary separates the two Tasman Sea regions. To the north the total sediment blanket is thick and the near absence of internal reflectors indicates a restricted environment of hemipelagic sedimentation characteristic of an enclosed sea with limited sediment provenances and few oceanic circulation changes.

Continental reconstructions based on magnetic lineation data for the Tasman Sea and South-east Indian Ocean lead us to the conclusion that unrecognized post-Cretaceous plate motions must have occurred between Lord Howe Rise and the Campbell Plateau or between East and West Antarctica. These motions are conjectural, but we favour a hypothesis based on motion across a hypothetical Antarctic "sphenochasm" or tectonic discordance lying along the structural boundary between East and West Antarctica. Such motions would have important ramifications in unravelling the geologic and tectonic history of West Antarctica.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Solar Neutrinos, Martian Rivers, and Praesepe

THE detection rate of solar neutrinos is significantly below the value predicted by contemporary theories of solar structure and evolution[1,2]. The most likely—or at least best advertised—explanation of the discrepancy is episodic and probably periodic core expansion in the Sun[3]; in the models developed to date, mixing of ^{3}He, produced by the p–p process, is the initiating event[4–7]. Although the radiative transfer time scale of photons from the core to the photosphere of the Sun is long, all the models identify epochs of anomalously low neutrino flux with epochs of anomalously low surface luminosity; and the temptation to connect the present "ice age" on Earth with low solar luminosity, L, has been unsuccessfully resisted. The peak-to-peak variations in L, $\Delta L/L$, range from about 7% to about 35% in these models, enough to have significant climatological consequences (see, for example, ref. 8). The duration of the variation is roughly the Kelvin–Helmholtz time scale $\sim 10^7$ yr; to correspond to the principal glaciation period, the interval between mixings must be $\sim 2.5 \times 10^8$ yr.

At present, conditions on the planet Mars are clearly those of an ice age climatology. A suggestion that much more clement conditions periodically recur on Mars[9] has seemingly been confirmed by the widespread presence of sinuous dendritic channels, sharply concentrated towards the planet's equator (refs. 10, 11 and unpublished results of C. S., O. B. Toun and P. Gierash). Many of these channels can be understood only in terms of flowing surface liquid water. The higher pressures, temperatures, and water abundances required to excavate these channels point to more Earth-like epochs on Mars. A general discussion of climatic instability on Mars (C. S. *et al.*, unpublished results) identifies several possible mechanisms; one of them is $\Delta L/L$ variations of 10 to 20%, with the present epoch near the solar luminosity minimum. Because of the permanent presence of oceans on Earth, and the fact that no significant atmospheric constituent condenses at the surface of the Earth, it is possible that climatic excursions on Earth are more damped, for a given $\Delta L/L$, than on Mars.

Such luminosity variations, if they occur, are unlikely to be restricted to the Sun. Other evolved main sequence stars should experience similar fluctuations. The colour-magnitude diagrams of open and globular clusters might, in principle, be used to test for such $\Delta L/L$. Were the effects of ^{3}He mixing to move a star exactly parallel to the main sequence, there would be no observable consequence of such $\Delta L/L$. But at least some of the published stirred models move stars at an angle to the zero age main sequence, and the width of the observed main sequence may therefore provide evidence on the existence and magnitude of such $\Delta L/L$.

Cluster colour-magnitude diagrams provide the obvious test because age and composition differences should not appreciably broaden the main sequence of an individual cluster. The cluster must not be too distant, otherwise the photometric errors will be excessive owing to faintness and crowding, and differential reddening across the cluster may become significant. For this reason all globular clusters, and such old open clusters as M67, NGC188 and NGC752, are excluded. On the other hand, the cluster must be sufficiently old to have produced ^{3}He in the cores of its solar-type stars. Also, the cluster stars must be older than the time required to produce the first core-mixing cycle—possibly several $\times 10^8$ yr for stars near 1 $M_\odot$. Finally, there must be an adequate number of stars not much more massive than 1 $M_\odot$, because it is the p–p cycle which produces ^{3}He rather than the C–N cycle (although C–N luminosity instabilities have not been excluded).

Open clusters such as the Pleiades are possibly near enough, but they are too young. Such clusters as the Hyades, Ursa Major, and Coma Berenices clusters may be sufficiently evolved, but they are too close—they subtend several degrees, and the apparent magnitude difference between the front and back of such clusters contributes significant scatter in the colour-magnitude diagram. (The Ursa Major cluster may be just close enough to separate out this effect through improved trigonometric parallaxes.)

The two remaining open clusters which may prove useful for our purpose are M39 and Praesepe. M39, however, has only about twenty stars and a visual extinction of 0.2 mag. Praesepe suffers none of these difficulties. At a distance of 159 pc it has no measurable reddening. It comprises ~ 100 stars, enough for meaningful statistics, and its turnoff age is $\sim 3 \times 10^8$ yr, probably old enough for our purpose.

The low luminosity end of the Praesepe colour-magnitude diagram has not evolved far enough to encounter a single mixing event. The stars at that end should be close to the zero age main sequence, and the scatter in the low luminosity main sequence should be representative of the observational errors. But more luminous stars, within a few magnitudes of the red giant turnoff, are sufficiently evolved to have experienced such mixing, if it occurs.

The photoelectric photometry of Praesepe was performed by Johnson[12], who, notably, remarked that the main sequence appeared distinctly wider for Praesepe stars earlier than about spectral type GO than for later types. He quotes as an upper limit to the intrinsic scatter of the later type main sequence a probable error of ±0.03 mag, corresponding to a standard deviation of ±0.05 mag. From Johnson's data we find a standard deviation for the main sequence stars in Praesepe between $(B-V)=0.3$ and 0.6 of about ±0.1 mag. Both estimates attempt to exclude double stars far above the main sequence. Thus, the square root of the differences between the upper and lower main sequence variances is nearly 0.1 mag in the correct sense: the more evolved stars show more scatter. (This estimate includes Johnson's quoted observational probable error of ±0.022 mag.) With a dispersion of 0.1 mag in the evolved main sequence of Praesepe, the total excursion in luminosity of individual evolved stars is $\sim 15\%$. In fact the total $\Delta L/L$ of an individual star will be somewhat greater, because the excursions in the colour-luminosity diagram have a component parallel to the main sequence which is undetectable by this method. Thus the Praesepe colour-magnitude diagram implies that solar type stars experience luminosity excursions of just the order apparently required to resolve the solar neutrino and Martian river problems.

The colour-magnitude diagrams of other evolved clusters give dispersion of this order or larger, but the observational errors are also larger. Thus cluster photometry is consistent with, but not necessarily uniquely indicative of, substantial variability in the solar luminosity on time scales $\sim 10^8$ yr. The fact that the Praesepe stars are approximately uniformly distributed through the width of the main sequence suggests that the excursion time off the main sequence is comparable with the time between mixings. Improved observation of Praesepe —and, possibly, M39 and the Ursa Major cluster—can shed further light on this problem. To the extent that climatic change caused by major solar variability produced major evolutionary advances on Earth, comparable biological evolution could have occurred on all other inhabited planets in the Galaxy.

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Effect of Low-level Radioactive Silver on Photographic Emulsions

LINDNER *et al.*[1] described the detection of low levels of radioactivity in silver bullion bars. Because this silver may be used in the manufacture of photographic emulsions the effects of the radioactivity are obviously of concern to photographic film manufacturers. We consider here the storage of photographic materials containing silver of the radioactivity level (10^{-4} μCi/g silver) given by Lindner *et al.*

We illustrate this point by calculating the rate of fogging that would result in a highly sensitive X-ray film for materials testing. Typically, such a film will be coated with about 2.3 mg of silver cm^{-2}. The silver bromide grains will have a diameter of about 1.5 μm. If such a film is irradiated with about 80 mR of γ-rays (0.3–2 MeV) an increase in optical density of about 0.3 will be the result. In a normal environment background, dose rate from cosmic radiation and radioactivity in the surroundings will be 80–100 mR yr^{-1}. Film manufacturers and users will rule out a film which, during storage, has obtained a fog level of optical density 0.3.

The film we describe will have about 3.5×10^8 grains cm^{-2}. With 10^{-4} μCi g^{-1} silver one will have a radioactive decay in 2.7×10^5 grains cm^{-2} /yr^{-1}. Thus about one per thousand of the grains will be directly involved in each year. If every radioactive decay involved one grain only, there would be no serious fogging, but the question "How many are involved ?" is not a trivial one.

As a first step, the decay of $^{110\text{m}}$Ag can be simplified. We will assume: for βs 0.087 MeV (61%), 0.529 MeV (36%) and 1.5 MeV (0.6%); for γs 0.67 MeV (127%), 0.88 MeV (139%) and 1.4 MeV (38%). The 0.087 MeV β-rays will only involve one grain. The amount of silver involved with the 0.529 βs we will leave open.

To calculate the effect of the γs we assume that the film is stacked with fifty sheets per cm thickness. If film and polyester film base were to be ground together, we would get a substance with a density of about 1.5 g cm^{-3} and with absorption coefficients μ_s of 0.105 cm^{-1} (0.67 MeV), 0.090 cm^{-1} (0.88 MeV), and 0.072 cm^{-1} (1.4 MeV). The radioactivity of this substance would be 0.43 d.p.s. per cm^3, and result in a photon emission S_v of 0.56 s^{-1} cm^{-3} (0.67 MeV), 0.61 s^{-1} cm^{-3} (0.88 MeV) and 0.167 s^{-1} cm^{-3} (1.4 MeV). The photon flux in the centre of an infinite slab source of homogeneous activity is

$$\varphi = B\, S_v[1 - E_2(\mu_s d)]/\mu_s$$

where B is the build-up factor, $E_2(\mu_s d)$ is the exponential integral of the second order and the thickness of the slab is $2d$. If d is about 15 cm, $\mu_s d$ will be >1; $E_2(\mu_s d)\sim 0.1$, so that $[1-E_2(\mu_s d)]$ can be neglected. Neglecting the build-up factor for the time being we obtain

$$\varphi \sim S_v/\mu_s$$

and with the data given we obtain photon fluxes of 5.3 s^{-1} cm^{-2} (0.67 MeV); 6.8 s^{-1} cm^{-2} (0.88 MeV); and 2.3 s^{-1} cm^{-2} (1.4 MeV) to give dose rates of 7×10^{-6} R h^{-1} (0.67 MeV); 12×10^{-6} R h^{-1} (0.88 MeV); and 6×10^{-6} R h^{-1} (1.4 MeV). Adding these fluxes gives a dose rate of 210 mR yr^{-1}. This result is obtained without taking a build-up factor of, perhaps, 2–5 into consideration. Strong fogging in this case is evident.

Workers requiring very sensitive emulsions must hope that the case described by Lindner *et al.* will be an isolated incident. Or will we have to purchase low-level counting equipment for raw materials control ?

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Radioactive Silver in East European Silver Bars

THE discovery of slightly radioactive silver in the form of the isotopes $^{108\text{m}}$Ag and $^{110\text{m}}$Ag in East European silver bars[1] is of considerable interest, because so far as is now known these short-lived radioactive isotopes have not been observed in natural materials[2]. Lindner *et al.*[1] have given four possible explanations for the presence of the radioactive isotopes, one of which is that the silver ore was mined by an underground nuclear explosion. An alternative natural explanation for the phenomenon seems more probable.

Some of the silver deposits in Eastern Europe contain uranium, chiefly as pitchblende. Most of these are veins and not particularly amenable to nuclear mining. Those in the Bohemian massif (Jáchymov) are well known and there are others in the Přibram area of Czechoslovakia and in the Tertiary and older rocks in the great metalliferous belt that extends through a number of Eastern European countries to the Black Sea.

The production of the $^{108\text{m}}$Ag and $^{110\text{m}}$Ag isotopes is essentially caused by slow neutron bombardment, the neutron capture reaction involving ^{109}Ag and ^{110}Ag being

$$^{109}\text{Ag}\ (\text{n},\gamma)\ ^{110\text{m}}\text{Ag}$$

In uraniferous silver deposits there are several neutron sources. Some of these are the various (α,n) reactions involving alpha particles from uranium and its decay products and various light elements such as Li, Be, B, and F, all of which occur often in some quantity in uraniferous silver deposits. There is also a source of neutrons in the spontaneous fission of uranium. According to Segrè[3] and Littler[4] the number of neutrons spontaneously emitted from natural uranium is $1.5\text{–}1.65\times10^{-2}$ g^{-1} s^{-1}. This is a relatively low neutron density, but probably sufficient to produce low levels of ^{108m}Ag and ^{110m}Ag in silver ores. When one considers the radioactive equilibrium conditions, the intimate contact of silver and uranium minerals in certain uraniferous silver deposits and the concentration factor (>5,000) inherent in winning the silver from the ore, it seems probable that both ^{108m}Ag and ^{110m}Ag would be concentrated in the silver bars in measurable amounts.

To test this speculation, I suggest that a search be made for small concentrations of ^{108m}Ag and ^{110m}Ag in silver minerals from uraniferous silver deposits (Great Bear Lake, Canada ; Jáchymov, Czechoslovakia) by well equipped laboratories using appropriate detection apparatus.

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Near-bottom Thermocline in the Samoan Passage, West Equatorial Pacific

ANTARCTIC bottom water (ABW) flows into the Pacific basin from the region between Macquarie Island and Antarctica[1-3]. Dynamic considerations suggest that the bottom water flows northwards as an intense current along the western boundary of the Pacific basin[4,5]. Oceanographers have noted that the Pacific is divided into at least four principal basins which are separated by relatively shallow sills that constrain the flow of water below 4,000 m (refs. 3, 6, 7). The narrow Samoan Passage, first recognized by Reid[8], is perhaps the most important channel with depth sufficient to allow a significant flow of bottom water from the southern basin into the central basin. Bottom current velocities in the passage of 5 to 15 cm s^{-1} towards the north have been measured[9] and Nansen casts in the passage revealed a sharp decrease in temperature of 0.27° C in 150 m at a depth of about 4,400 m[10]. This benthic thermocline marks the abrupt boundary between the deep water and the ABW and supports the observation of cold bottom water flow through the passage. (In a personal communication, P. Lonsdale and J. Reid suggested that the bottom water may include entrained high salinity Atlantic deep water as well as ABW.)

In the autumn of 1971, thirteen temperature profiles were taken with a thermal gradient recorder mounted on a sediment corer in and near the Samoan Passage in order to define the flow of bottom water through the area. The resistance of a precisely calibrated thermistor attached to the top of the instrument was recorded every 30 s as the corer was lowered to and raised from the bottom. A pinger was mounted 78 m above the core weight so that a time record of the depth of the instrument could be recorded aboard ship. The temperature time data recorded on film in the heat flow instrument were then merged with the depth/time data to yield *in situ* water temperature profiles. The relative precision of the thermistor was 0.002° C and the absolute accuracy about 0.01° C. The error in depth determination was ~20 m near the bottom but could be as much as 150 m at a distance of 1.5 km above the bottom because of rapid changes in bathymetry and variations in the rate of film speed in the thermal gradient recorder. This estimate of the error in the depth determination is in agreement with the magnitude of discrepancies in the temperature profiles obtained from the lowering and subsequent raising of the instrument at the same station.

A pronounced benthic thermocline was present in all eight of the new temperature profiles taken in the axis of the passage (Figs. 1 and 2). A temperature decrease of 0.31° C (1.37° C to 1.06° C) occurs over a depth interval of about 250 m at a depth of 4,200 to 4,400 m. Below 4,400 m there is a slight increase in temperature because of adiabatic compression. The thickness of the ABW using the 1.10° C isotherm (*in situ*) is 800–950 m in the axis of the passage (Fig. 2). The thickness of the layer decreases near the walls of the passage in such a way that the boundary between the deep water and the bottom water (about 1.10° C) remains at a depth of about 4,200–4,400 m. At the northern end of the passage, the depth of the benthic thermocline increases while the thickness of the bottom water layer decreases (Fig. 2). The benthic thermocline is not clearly present in four of the five temperature profiles taken north of the passage. The profiles do show a slight decrease in temperature (0.05° C, see northernmost temperature profile in Fig. 1) and a warmer isothermal layer. The sharp boundary between the bottom and deep water may be obscured by turbulence as the water flows over the sill to the north (P. Lonsdale, personal communication).

The temperature data suggest that the wedge of bottom water flowing northwards is narrowest and thickest in the axis of the passage (Fig. 2). During Chain 100, two current meters were placed in the axis of the passage (8° 15′ S,

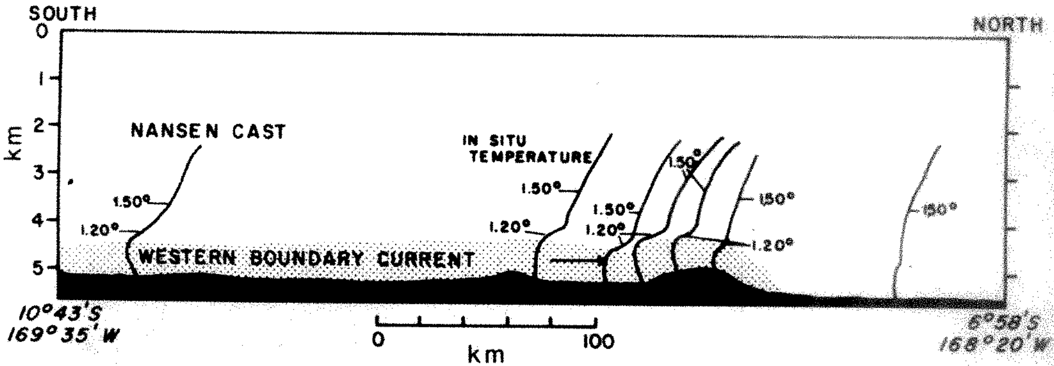

Fig. 1 Benthic thermocline in the Samoan Passage. The upper boundary of the western boundary current is taken to be the 1.10° C (*in situ*) isotherm. The current fans out and thins north of the passage. (Core head thermograd, RV Chain.)

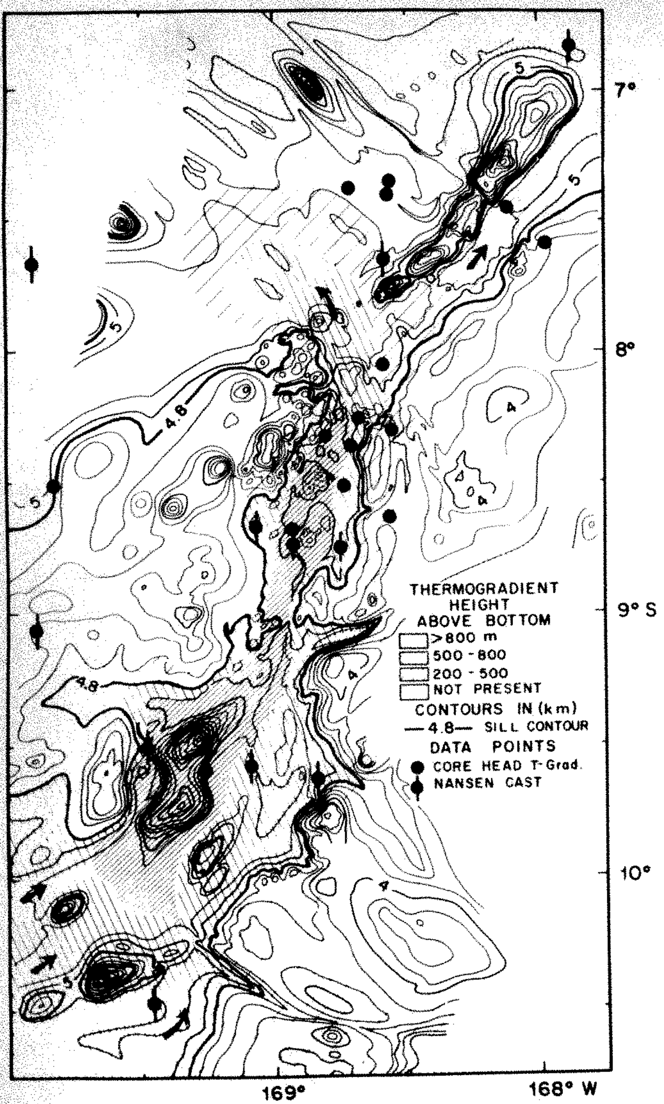

Fig. 2 Isothermal layer thickness in Antarctic bottom water. The bottom water layer is thickest in the narrow axial zone of the passage. North of the passage the flow bifurcates, with most of the water flowing to the north-west. The bathymetry was contoured by P. Lonsdale based on data from the Chain 100 and Styx expeditions. Recently acquired data indicate that the north-west channel may be narrower than shown.

168° 43′ W, and 8° 16′ S, 168° 40′ W) for 139 h and 25 h, respectively, at a distance of 3 m from the bottom. A mean velocity of 9.3 cm s^{-1} in a northerly direction was recorded, with a maximum velocity of 16 cm s^{-1}. Using a topographic cross-section at 8° 30′ S, and an average velocity of 9.3 cm s^{-1}, we derive a transport of about 3 Sverdrup (10^6 m^3 s^{-1}) of cold bottom water flowing from the southern basin into the central basin of the Pacific.

North of the passage, a topographic high creates two channels, one striking north-east, the other north-west. Temperature data show a 320 m thick layer of bottom water in the axis of the north-easterly passage. A current meter placed near the east side of the channel for 3 d recorded a mean velocity of 2 cm s^{-1} with a maximum of 4.9 cm s^{-1}. But the direction of flow was north-east for 1.5 d and south-west for 1.5 d, so the net flow is near zero. A more constant northerly flow may exist in the axis of the north-east channel. The lack of a sharp benthic thermocline in the four temperature profiles near the topographic high north of the passage, and the low flow through the north-easterly channel, suggest that most of the flow (about 3 Sverdrup) is directed towards the north-west. The greater depth of the north-west channel as well as westward deflexion due to Coriolis forces (although weak at this latitude) would favour a greater flow to the north-west.

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Changes in the Isotopic Composition of East Mediterranean Seawater during the Holocene

WHEN molluscs secrete their shells, the $\delta^{18}O$ in the shell is a function only of the temperature and of the $\delta^{18}O$ in the water[1-4]. Thus one can compute the temperature at which an ancient shell was formed by measuring its $\delta^{18}O$ value if one assumes that of the water in which it grew. For Pleistocene samples there has been a heated discussion as to the degree of change in the $\delta^{18}O$ of the sea[5-7].

The present Eastern Mediterranean (EM) is more saline[8] and has a higher $\delta^{18}O$ than the Western Mediterranean (WM). Here we consider whether this difference always existed and, if not, when it occurred. We measured the oxygen isotopic composition and ^{14}C ages of samples of the aragonite forming *Glycimeris violacescens* from several locations (present and fossil shorelines) along the Israeli coast (Fig. 1).

The geological setting of these samples will be described elsewhere. Each shell specimen was cleaned by washing and by boiling in H_2O_2. This was followed by grinding and X-ray diffraction analysis to ascertain that no recrystallization to calcite had occurred. Carbon dioxide was extracted by the method of McCrea[9] and duplicates were run in a double collecting Atlas M-86 mass spectrometer. The reproducibility on duplicate analysis was 0.02%.

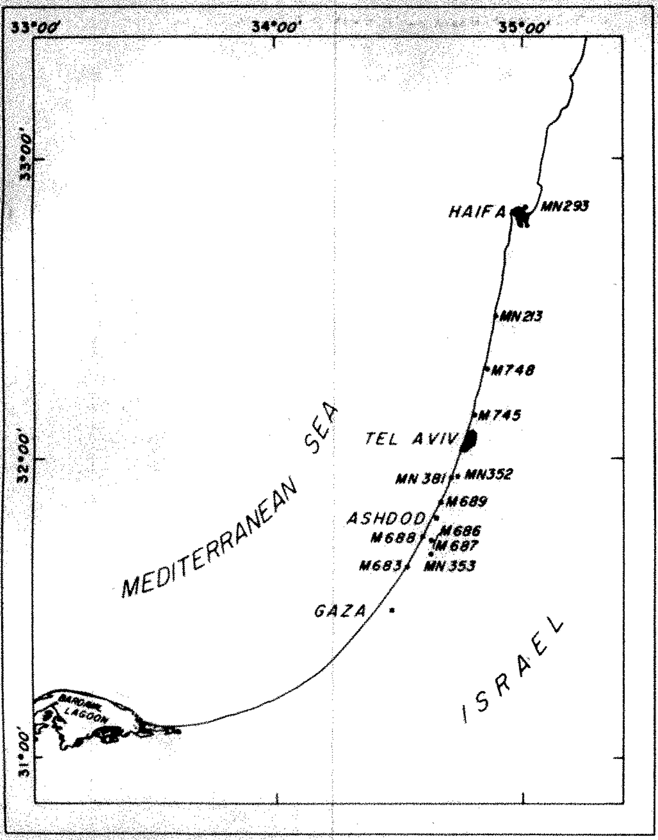

Fig. 1 Map of the coast of Israel showing locations where *Glycimeris* shells were collected for ^{18}O and ^{14}C analyses.

Carbon-14 analyses were performed as described by Carmi *et al.*[10]. Because such an analysis requires up to thirteen specimens and ^{18}O analyses are performed on single specimens, we use the term "sample" to refer to the entire group analysed from any location, although ^{18}O was determined for every specimen in the sample.

Table 1 shows the location, number of specimens analysed in each sample, ^{14}C age, average δ^{18}O and standard deviation in δ^{18}O for the specimens of each sample. In nearly half of the cases this standard deviation is equal to the analytical error. We encountered one sample (from a submarine drill core off Nahal Alexander) which had so large a deviation (0.93) that it was obviously a mixture of populations. For this reason no ^{14}C analysis was performed on it.

Because the present δ^{18}O of the EM is +0.18% (SMOW), from the equations of refs 3 and 4, we calculate the temperature for sample MN293 to be 25° C. This is equal to the average spring to autumn temperature for Israel coastal waters[11]. (Molluscs generally secrete their shells in this period.)

Table 1 Ages and δ^{18}O Values of the Samples Analysed

Sample No.	Location	No. of specimen	Age (yr)	δ^{18}O% Average	δ^{18}O% Standard deviation
MN293	Haifa	3	0	−0.014	0.005
MN394	Principality of Monaco	3	0	−0.16	0.020
MN381	Palmachim	9	1,150	0.00	0.053
M688	Ashdod	9	1,500	0.016	0.028
M686	Ashdod Yam	10	2,120	0.018	0.037
M687	Ashdod Yam	13	2,360	0.041	0.024
M683	Ashkelon	6	2,610	0.033	0.010
M745	Tel Baruch	6	2,920	0.015	0.030
MN213	Caesaria	8	3,300	−0.050	0.014
M689	Rogozin	5	3,800	0.04	0.020
M748	Nahal Poleg	8	3,800	−0.070	0.020
MN352	Nahal Soreq	8	4,600	−0.078	0.040
MN353	Nitzanim	6	4,900	−0.147	0.045

In Fig. 2*a* we show an overall spread of 0.19% in the δ^{18}O of the population averages. According to the Craig equation[8] such a spread must be due to variations in the temperature and/or the δ^{18}O of the water. Thus it can be explained in one of the three following ways:

(1) By assuming that δ^{18}O in the water was always what it is today. Fig. 2*b* shows the temperatures calculated from this assumption. We conclude that this assumption is very improbable because of the large temperature range (9° C) over this time interval. Other evidence suggested that the temperature range for this interval is no more than 2° C (ref. 12).

(2) By assuming that the water temperature has not changed. Fig. 2*c* shows the δ^{18}O of the water as calculated from this assumption. But Emiliani *et al.*[1] show that temperature variations have occurred in the WM.

(3) By assuming that water temperatures in the EM have varied in the same way as in the WM[1] but that there was always a 4° C difference between EM and WM (which is the difference today). Thus neither temperature nor δ^{18}O of the water remained constant. Figure 2*c* shows the δ^{18}O of the water calculated on this assumption.

We have shown that the first two hypotheses are improbable so we are inclined to accept the third one. The consequences are that the δ^{18}O remained approximately at the present value for the past 2,800 yr, and that between 2,800 and 4,900 yr BP there was a change in δ^{18}O of the EM water from +0.2 to +0.1% (SMOW). We note that the value of δ^{18}O calculated for EM water 4,900 yr BP is similar to that of present WM (0.1%).

How can this change have arisen? It is well known that in large seas high δ^{18}O values are accompanied by high salinities. This is explained as the consequence of increases in both parameters as a result of an excess of evaporation over precipitation. At present there is a net excess of evaporation over precipitation of 0.8 m.y.$^{-1}$. Thus, our data imply that no such excess existed in the EM 4,900 yr BP.

We do not know what may have caused so sharp a change

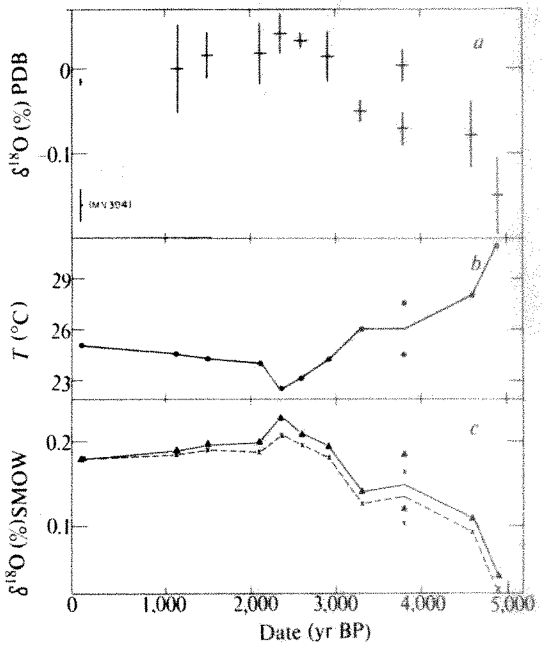

Fig. 2 *a*, Observed ^{18}O as a function of age for the *Glycimeris* samples from Israel. The point labelled MN394 is from Monaco and is shown for purposes of comparison. *b*, Calculated temperatures as a function of age for the samples shown in Fig. 2*a* based on assumption (1). *c*, Calculated water ^{18}O as a function of age for the samples shown in Fig. 2*a*. △, Based on assumption (2); ×, based on assumption (3).

in the evaporation–precipitation balance in the EM 4,900 yr BP. It seems that the small temperature change detectable would be insufficient to explain this. One possibility is a change in wind direction. Another possibility is a tectonic change which caused a rise in the sill of the Sicilian strait and impaired the contact between the EM and the WM. Supporting evidence for a change from humid to dry climate at about 5,000 yr ago is found in palynological studies of the Northern Mediterranean region[13] and of Northern Israel[14].

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Recent Rainfall Trends in Africa, the Middle East and India

IN the Middle East and Africa north of the Sahara the wet season extends from October to May; small amounts of rain also fall in Pakistan and north-west India at that time of year. Winter–spring rainfall in these areas is controlled primarily by divergence patterns in pressure troughs in the mid- and upper-tropospheric circumpolar westerlies. Rainfall is associated with the widespread ascent of warm, moist air from the tropics on the eastern side of the troughs, and also with deep convective instability in the cold air at, and slightly to the rear of, the trough axes, especially over coasts and mountains. It is in winter that the increase in the latitudinal temperature gradient in the northern hemisphere causes the westerly circulation to increase in strength and the westerly troughs to penetrate into sub-tropical latitudes. In summer, the latitudinal temperature gradient is at a minimum, the general circulation is relatively weak, and the westerly troughs are of small amplitude and restricted to middle and high latitudes.

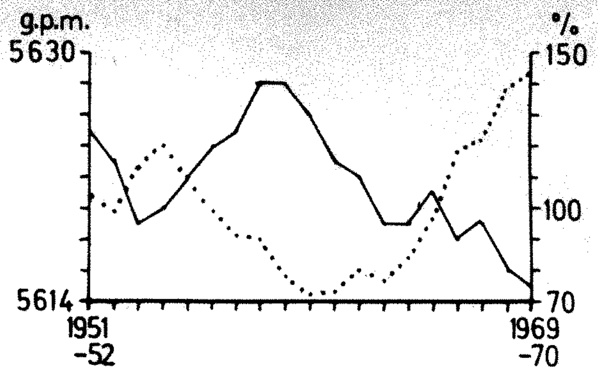

Fig. 1 . . . . , Five-year running means of the mean percentage of normal seasonal rainfall (October to May) at Bikaner (India), Shiraz (Iran), Mosul (Iraq), Beirut (Lebanon), Jerusalem (Israel), Jiddah and Riyadh (Saudi Arabia), Nalut (Libya), Marrakech (Morocco) and Atar (Mauritania); ——, five-year running means of the mean altitude of the 500 mbar surface (geopotential metres) along 40° N, between 110° W and 70° E, from October to May (data extracted from *Die Grosswetterlagen Europas* of the German Weather Service (1949–1972)).

In order to study recent rainfall trends over North Africa, the Middle East and north-west India, seasonal totals from ten widely separated stations (Fig. 1) were used, with mean values ranging from 24 to 893 mm. Figure 2 shows that the rainfall trends, although somewhat out of phase, are similar at widely separated stations with vastly different rainfall totals. The seasonal rainfall at each station was expressed as a percentage of the normal, and the mean percentage of all the stations determined, thus giving a crude area–mean seasonal rainfall figure. Smoothing was achieved by computing five-season running mean values (Fig. 1). The trend from the mid-1950s to the early 1960s was towards lower seasonal rainfall, but the trend has since been completely reversed.

Winter–spring rainfall in these sub-tropical regions is particularly sensitive to the southward extent of the troughs in the mid-tropospheric circumpolar westerlies. Thus, the altitude of the 500 mbar surface along 40° N is a meaningful index of the southward extent of the westerly troughs for analysing rainfall deviations (my unpublished work). The five-season running means of the mean altitude of the 500 mbar surface along 40° N, between 110° W and 70° E, from October to May are shown in Fig. 1.

Figure 1 shows that, since 1951/52, the altitude of the 500 mbar surface along 40° N and the winter–spring rainfall over north Africa, the Middle East and north-west India have been highly negatively correlated: the correlation coefficient is $r = -0.74$. The decrease in the altitude of the 500 mbar surface during the 1960s signifies an increase in the southward extent of the troughs in the circumpolar westerlies. In the late 1960s the seasonal rainfall totals over large areas of north Africa and the Middle East were the highest ever recorded.

This tendency, towards northerly meridionality in the general circulation of the northern hemisphere, has also been reflected in an excessive amount of sea ice in the European sub-Arctic, and a decline in the frequency of westerly winds over Britain[1]. There are also repercussions in the summer monsoon rainfall areas to the south of the Sahara and in south-west Asia, where

Table 1 Five-year Running Mean Percentage of Normal Seasonal Rainfall Centred on 1957 and 1970

Country Station	India Bikaner	India Jodhpur	Sudan Khartoum	Niger Agades	Mali Tessalit	Mali Gao	Mauritania Nouakchott	Mauritania Atar	Mean
1957	114	115	122	130	140	114	106	121	120
1970	71	68	80	44	63	75	74	52	66

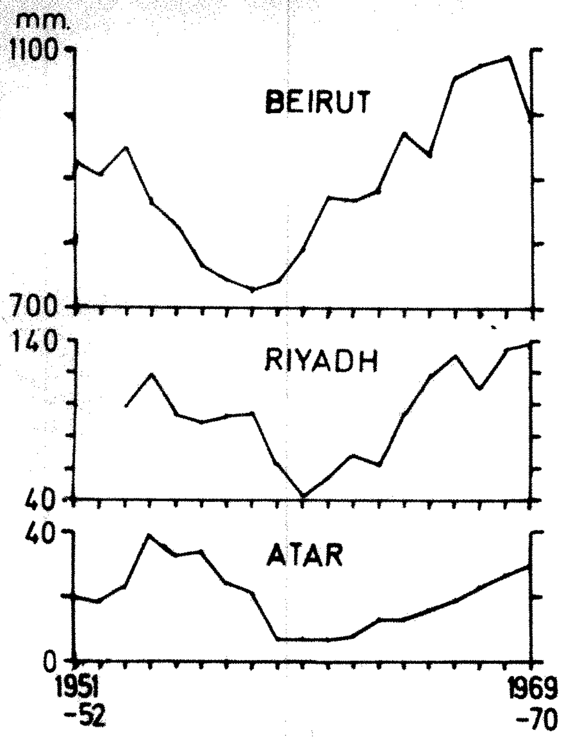

Fig. 2 Five-year running means of seasonal rainfall (mm) at Beirut (Lebanon), Riyadh (Saudi Arabia) and Atar (Mauritania).

the synoptic scale systems controlling rainfall are quite different from those mentioned above. From Mauritania across to north-west India the summer monsoon rainfall has decreased steadily by more than 50% since 1957; Table 1 shows the five-year running mean percentages of normal seasonal rainfall centred on 1957 and 1970 for eight stations towards the northern limit of the monsoon rains. These are the areas where rainfall is marginal for agriculture and where the failure of the monsoon rains can be economically disastrous. There have been press reports recently of widespread and severe drought in western India, Chad, Niger, Upper Volta, Dahomey, Mali, Senegal and Mauritania. It is not unusual for the monsoon rains to fail in some of these regions each year, but it is almost unknown for them to fail so widely in several consecutive years. It is probable that the abnormal southward extent of the troughs in the circumpolar westerlies has restricted the northward extent and intensity of the tropical circulation systems controlling the monsoon rainfall, and also the mean meridional circulation of the Hadley Cell. A similar relationship between large-scale eddies in the circumpolar westerlies and the vertical motions in the Hadley circulation has been found in laboratory experiments[2]: with short waves in relatively weak circumpolar westerlies, a meridional circulation was evident in the tropics; but as the circumpolar westerlies expanded the tropical meridional circulation broke down.

The results provide further evidence of recent changes in the general circulation of the northern hemisphere and the equatorward shift of climatic zones; a trend which was first evident in the 1930s (ref. 3), but which became even more marked in the 1960s.

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BIOLOGICAL SCIENCES

Catecholamines in the Median Eminence: New Evidence for a Major Noradrenergic Input

THE median eminence of the hypothalamus contains large amounts of hypothalamic hormones (releasing factors)[1], but the cellular localization of these hormones is unknown. In contrast, there is convincing evidence, from fluorescence histochemical studies, for the existence of a dopaminergic system with neuronal cell bodies in the arcuate nucleus, whose axons project into the external layer of the median eminence[2]. Deafferentation studies combined with fluorescence[3,4] or electron microscopy[5] have suggested that there may also be a noradrenergic component in the innervation of the median eminence. We now report additional biochemical evidence supporting the existence of a substantial noradrenergic input to this brain area. We also describe the properties of a dopamine (DA) uptake system in the median eminence.

Wistar male rats weighing 150–250 g were used. The median eminence was removed using a stereomicroscope. The pia mater was peeled off and the median eminence removed by dissecting the lateral and frontal borders (defined by the presence of capillary loops) while holding the stalk with fine forceps; the stalk was then removed. The average weight of such samples was approximately 0.3 mg. To excise the arcuate nucleus two coronal cuts were made in the remaining hypothalamus, the frontal cut at the level at which the optic tracts enter the brain, and the second at the premammillary area. From these sections a triangular shaped tissue sample about 1 mm wide and 1 mm high was obtained from the medial and basal hypothalamus. The average weight of such samples was 1.5 mg.

The catecholamine content of the tissue was determined in pools of four to ten median eminences or arcuate nuclei homogenized in 15–20 vol of 0.1 M perchloric acid. Noradrenaline (NA) and DA were assayed in duplicate aliquots of the homogenate by sensitive and specific radiochemical enzymatic techniques. The technique for NA was a modification of that described by Iversen and Jarrott[6] which exploits the phenylethanolamine N-methyl transferase (PNMT) catalysed methylation of tissue NA to adrenaline in the presence of ^{3}H-methyl-S-adenosyl methionine. Dopamine estimation involved catechol-*O*-methyl transferase (COMT) catalysed methylation of this amine to tritiated 3-methoxytyramine (unpublished results of A. C. C. and L. L. I.). In each assay the labelled products were separated by paper chromatography, eluted and the radioactivity counted. The amounts of catecholamines in tissue extracts were determined by comparison with NA and DA standards. Using the PNMT method we measured as little as 1 ng of NA, and with the COMT method, as little as 2 ng of DA. Confirming the results from fluorescence microscopy[2], the concentration of DA in the median eminence was found to be very high (Table 1), comparable with that measured by this method or previously reported by fluorimetric assay procedures for the corpus striatum[7]. The DA content of the arcuate nucleus tissue, however, was below the sensitivity of the method (0.5 μg g^{-1}).

The results obtained with the PNMT method for NA in median eminence were unexpectedly high (Table 1). The individual values ranged from 2.7 to 5.4 μg g^{-1} tissue, concentrations higher than previously reported in any other brain region. The concentrations of NA and DA, when assayed in parallel aliquots from the same tissue extracts, were similar. The possibility that the high NA values in the median eminence might have arisen through *in vitro* conversion of DA to NA, and thence to adrenaline in the PNMT reaction is considered unlikely because NA estimations in the DA-rich corpus striatum with the same method failed to give values above the blank level.

A specific uptake mechanism for DA exists in the DA terminals of the corpus striatum[8], and it was thus of interest to determine whether a similar mechanism exists in the median eminence. The fluorescence histochemical studies of other workers[9,10] have suggested that catecholamine uptake does occur in terminals in the tubero-infundibular region. A modification of the method of Horn and Snyder[11] was used to study the uptake process in homogenates of pooled median eminence and arcuate nucleus tissue. Uptake of catecholamines into brain homogenates occurs predominantly into synaptosomes[12]. Synaptosomes form when median eminence tissue is homogenized, and a recent study[13] has demonstrated the *in vitro* release of amino-acids and releasing factors from such preparations.

Table 1 Noradrenaline and Dopamine Content of the Rat Median Eminence and Arcuate Nucleus

Tissue	Catecholamine	Concentration
Median eminence	Dopamine	10.3±0.98 μg g^{-1} (5)
Median eminence	Noradrenaline	4.1±0.53 μg g^{-1} (4)
Arcuate nucleus	Noradrenaline	1.3±0.12 μg g^{-1} (3)

The catecholamine contents of pooled median eminence and arcuate nucleus tissue were estimated by radiochemical enzymatic methods (see text). Results are expressed as the mean value ±s.e.m. with the number of experiments in parentheses.

Tissue was weighed and then homogenized in 50 vols of 0.25 M sucrose and samples of homogenate equivalent to 0.2–0.4 mg of original tissue weight were incubated at 37° C in a Krebs–Henseleit medium containing small amounts of ascorbic acid, EDTA and a monoamine oxidase inhibitor (nialamide) to inhibit the spontaneous or enzymatic breakdown of labelled catecholamine. ^{3}H-Dopamine (2-^{3}H, SA=3.48 Ci mmol^{-1}; NEN Chemicals Gmbh, Dreieichenhain, Germany) was present at a concentration of 1×10^{-7} M.

There was a rapid uptake of radioactivity giving an estimated tissue: medium ratio of up to 30:1 for the median eminence after incubation for 5 min at 37° C. The tissue: medium ratio is defined as d.p.m. g^{-1} of original tissue weight per d.p.m. ml.$^{-1}$ of incubation medium. The uptake process for ^{3}H-DA in the median eminence had the properties of an active transport, similar to those of the catecholamines in other brain areas[14,15]—it was temperature and sodium dependent (Table 2).

To explore the relative contributions of noradrenergic and dopaminergic neurones to this uptake we used drugs that have a selective inhibitory effect on the uptake of catecholamines into these two categories of nerve terminals. Desipramine, in particular, is about 1,000 times more potent as an inhibitor of the catecholamine uptake sites in noradrenergic nerve endings than in dopaminergic terminals[16,17]. This drug was used at a concentration of 5×10^{-7} M, which from previous experience would cause about 90% inhibition of NA uptake sites, while leaving DA uptake unaffected[16]. In contrast, benztropine is more selective in inhibiting catecholamine uptake into dopaminergic nerve endings[16].

Table 2 Effect of Sodium Removal and Low Temperature on Dopamine Uptake by Median Eminence Homogenates

Temperature	Medium	^{3}H-dopamine uptake as % control
37° C	Na$^+$ (control)	100 ±4.3 (4)
37° C	Na$^+$ free	37.5±3.1 (4)
0° C	Na$^+$	10.5±2.9 (4)

Effect of sodium-free medium (NaCl replaced by 0.25 M sucrose) and low temperature on the uptake of ^{3}H-dopamine (1×10^{-7} M) in Tris–Krebs medium (NaHCO$_3$ replaced by 5 mM Tris/HCl, *p*H 7.4) by median eminence homogenates. Results are expressed as the mean values±s.e.m. with the number of experiments in parentheses.

Table 3 Effect of Desipramine on ^{3}H-Dopamine Uptake

Tissue	^{3}H-dopamine uptake Control	DMI-% control
Median eminence	100.0±2.0 (4)	68.3±3.1 (4)
Arcuate	100.0±1.6 (3)	34.0±1.2 (3)
Pituitary stalk	100.0±6.5 (3)	63.0±8.4 (3)
Striatum	100.0±0.1 (6)	108.0±0.1 (8)

The effect of desipramine (5×10^{-7} M) on the uptake of ^{3}H-dopamine (1×10^{-7} M) by homogenates of median eminence, arcuate nucleus, pituitary stalk and striatum. Results are expressed in % of control as the mean value±s.e.m. with the number of experiments in parentheses. Mean values for ^{3}H-dopamine uptake in control experiments, expressed as tissue : medium ratios, were: median eminence=30; arcuate=29; pituitary stalk=92; and striatum=109.

In the arcuate-median eminence complex desipramine (5×10^{-7} M) produced a 36% inhibition of ^{3}H-DA uptake. When the arcuate nucleus, median eminence and hypophysial stalk were investigated separately desipramine was found to produce a more marked inhibition of ^{3}H-DA uptake in the arcuate nucleus than in the other two areas (Table 3). At this concentration it caused no significant inhibition of ^{3}H-DA uptake in the corpus striatum (Table 3). These results thus support the concept of a substantial noradrenergic input to the median eminence.

To investigate the properties of the uptake sites in dopaminergic neurones in the median eminence, without the complications of transport of ^{3}H-DA into noradrenergic terminals, the effect of various concentrations of benztropine on ^{3}H-DA uptake was investigated in the presence of 5×10^{-7} M desipramine. In such conditions, benztropine produced a dose-dependent inhibition of ^{3}H-DA uptake into the median eminence homogenates (Table 4). The IC50 value for benztropine of (9.0×10^{-8} M) was very similar to that previously obtained by Coyle and Snyder[18] for inhibition of DA uptake in homogenates of the rat corpus striatum.

Table 4 Inhibition of Desipramine-insensitive ^{3}H-Dopamine Uptake into Median Eminence Homogenates by Benztropine

Concentration of benztropine (M)	% Inhibition of H-dopamine uptake
1×10^{-6}	75.5±2.10 (4)
1×10^{-7}	63.3±6.74 (3)
5×10^{-8}	32.0±3.51 (4)
IC50=(9.0×10^{-8} M)	

Benztropine at three concentrations was preincubated for 5 min at 37° C with median eminence homogenates in the presence of desipramine (5×10^{-7} M). ^{3}H-Dopamine (1×10^{-7} M) was then added and the incubation continued for a further 5 min. Values for inhibition are the mean±s.e.m. with the number of experiments in parentheses. The IC50 is defined as the concentration of drug producing a 50% inhibition in substrate uptake. It was determined by a graphical method using log-probit paper[16].

Our results give more precise quantitative information on the catecholamine content of the median eminence of the rat than has previously been available, and these data indicate that in this area of the brain the ratio of DA to NA is close to 2:1. The values reported here for DA concentration in the median eminence are considerably higher (with one exception[19]) than those previously published[20–22]. This discrepancy may be because the present sensitive assay techniques allowed catecholamine estimations to be performed in relatively small, precisely dissected areas of hypothalamus. The less sensitive fluorimetric techniques previously used necessitate the pooling of large quantities (up to 100 median eminences per sample) of material which may have been contaminated by neighbouring structures of low catecholamine content.

The report from fluorescence histochemical studies, that catecholamines are taken up in the arcuate-median eminence complex[9,10], has been confirmed and additional quantitative biochemical data are provided. In addition we have demonstrated that there is a substantial desipramine-sensitive component in such uptake, which was not detected in the previous studies[10]. The demonstration of a benztropine-sensitive uptake mechanism for DA in tubero-infundibular neurones also supports the view that the uptake system in these dopaminergic neurones has properties similar to those found in other neurones in the CNS. The catecholamine concentrations in the median eminence, together with the uptake results, indicate that the noradrenergic innervation of this structure is considerably larger than was previously suspected. Both DA and NA have been implicated in the neuronal regulation of adenohypophysial secretion[23], but more information is needed for an understanding of their precise physiological roles in the median eminence.

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Amino-acid Substitution in the $\alpha^1\beta^1$ Intersubunit Contact of Haemoglobin-Camden β131 (H9) Gln→Glu

THE structures of more than 100 variant human haemoglobins have been determined and in many cases it has been possible to relate the position of the individual amino-acid substitution to the stability and function of the molecule[1,2]. Here we describe the amino-acid replacement, structural alterations and oxygenation properties of a variant, haemoglobin-Camden, which has a substitution in the $\alpha^1\beta^1$ intersubunit contact of the haemoglobin molecule.

The variant was discovered during routine starch gel electrophoresis of red cell haemolysates from antenatal patients of African extraction, to exclude the presence of sickle cell trait. Investigation of the patient showed no anaemia or evidence of haemolysis: haemoglobin, 11.1 g per 100 ml. of blood; packed cell volume 41%; red cells, 4.4×10^9 ml.$^{-1}$; mean cell volume 93 μm^3; MCHC, 27G per 100 ml.; reticulocytes, 0.1%. There was no splenomegaly. Oxygen dissociation studies on the patient's red cells suspended in isotonic phosphate buffer at 37° C (ref. 3) gave values that were within the normal range: $P_{50}=31.5$ mm Hg $n=2.7$ at pH 7.13; $P_{50}=22.0$ at pH 7.46; $P_{50}=44.0$ at pH 6.47; Bohr shift (pH 7.46–7.13) $= -0.47$.

On starch gel electrophoresis in Tris-EDTA-borate buffer, pH 8.6, the variant haemoglobin-Camden migrated just ahead but was poorly resolved from the normal Hb-A, whereas in phosphate buffer at pH 6.9 no separation of the two haemoglobins was observed. These results excluded the possibility that Hb-Camden was Hb-J, as the latter is clearly resolved from Hb-A in both these systems. The proportions of the haemoglobins in the patient's haemolysates determined by densitometry in the pH 8.6 gel system were Hb-Camden 45% and Hb-A_2 3.2%; the remainder was Hb-A. Complete separation of Hb-Camden and Hb-A was subsequently achieved by agar gel electrophoresis in citrate buffer, pH 6.2, the Hb-Camden migrating anodally to Hb-A.

As it proved difficult to isolate the variant haemoglobin by starch block electrophoresis or column chromatography, globin was prepared from the unseparated Hb-Camden and Hb-A. Chromatography of the globin on CM cellulose[4] showed an abnormal chain eluting just before the normal β^A chain, the position of elution suggesting a single negative charge change from the β^A chain. Tryptic peptide mapping of the aminoethylated[6] abnormal chain showed that βT13 was replaced by a more anodal peptide, the position of the new peptide corresponding to an extra negative charge on a peptide of that size (Fig. 1). After elution from the fingerprint, amino-acid analysis of this peptide showed the same

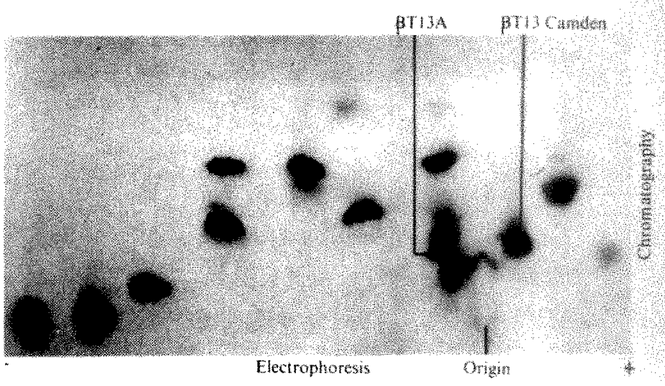

Fig. 1 Peptide map of the tryptic digest of the isolated β-chains of Hb-Camden. The normal βT13 peptide is replaced by a more anodic peptide. (A trace of β^A globin was present in this preparation.) Electrophoresis at pH 6.4 in pyridine : acetic acid : water (10 : 0.1 : 90). Chromatography in iso-amyl alcohol : pyridine : water (35 : 35 : 27) (ref. 5).

Table 1 Amino-acid Analysis of Hb-Camden βT13

	Molar ratios of amino-acids	
Amino-acid	Hb-Camden βT13	Expected values from Hb-A βT13
Thr	0.9	1
Glu	3.2	3
Pro	1.9	2
Ala	1.9	2
Val	1.2	1
Tyr	0.8	1
Phe	1.0	1
Lys	1.0	1

Hb-Camden βT13 was hydrolysed with 6.7 N HCl at 110° C for 16 h. The hydrolysate was applied to an amino-acid analyser.

molar ratios of amino-acids as would be expected from Hb-A βT13 (Table 1), indicating the replacement of one glutamine residue by glutamic acid. Two glutamine residues are, however, present in this peptide from Hb-A:

127 131
Hb-A βT13 Glu.Phe.Thr.Pro.Val.Gln.Ala.Ala.Tyr,Gln.Lys

To determine which glutamine residue was substituted, Hb-Camden βT13 was partially digested with carboxypeptidases A and B[7]. Subsequent reaction of the amino-acids released with dansyl chloride, followed by high voltage electrophoresis[8], showed the presence of Lys,Glu,Ala and traces of Gln and Asp. This result suggested that the more C-terminal, β131 Gln, was replaced by Glu. Further confirmation was obtained by digesting Hb-Camden βT13 with chymotrypsin, followed by peptide mapping which showed one very strongly staining neutral peptide as well as a number of more weakly staining spots. The strong neutral peptide was hydrolysed with HCl, after which reaction with dansyl chloride followed by high voltage electrophoresis showed the presence of only Glu and Lys and because it was neutral it must have contained equal amounts of these. The neutral character of the peptide indicated that glutamic acid, not glutamine, was present. The peptide Glu–Lys can only have arisen from the C-terminus of βT13 thus confirming that the substitution in Hb-Camden is β131 (H9) Gln→Glu.

In normal human haemoglobin-A Gln (H9) β131 forms part of the $\alpha^1\beta^1$ intersubunit contact by coordination with His (G10) α103 (ref. 9). In a private communication, Dr M. F. Perutz indicated that substitution of the glutamine residue by glutamic acid would be expected to result in formation of a salt bridge between the carboxyl group of the glutamic acid and the imidazole of the histidine. In Hb-A His (G10) α is in a rather secluded position facing the internal cavity on one side and cut off from water on the other sides. Moreover it is probably in contact with the amino rather than the carboxyl side chain of Gln (H9) β. Thus it would be expected to have a low *p*K and be uncharged at *p*H 8.6 and *p*H 6.9. In Hb-Camden, the formation of a salt bridge with the new glutamic acid would raise the *p*K of the imidazole so that it would now be charged at these *p*Hs and this extra positive charge would compensate the negative charge of the glutamic acid. Such interactions would explain that Hb-Camden has nearly the same electrophoretic mobility as Hb-A at *p*H 8.6 and *p*H 6.9.

The functional properties of Hb-Camden in the heterozygote appear to be normal, as no abnormalities in oxygen affinity, haem–haem interactions or Bohr shift could be detected and no clinical symptoms were observed. The effect of this substitution in the $\alpha^1\beta^1$ contact could not be studied further as the patient was no longer available.

Eleven human haemoglobin variants have been reported with substitutions in the $\alpha^1\beta^1$ intersubunit contact[10]. Of these six show no abnormal properties, three show only mild abnormalities and only two give rise to haemolytic anaemia as a result of molecular instability. These observations are in accordance with data on the normal haemoglobins of other animals, among which more than one third of the residues in the $\alpha^1\beta^1$ contact vary (in contrast to the residues in the $\alpha^1\beta^2$ contact which are virtually invariant)[9]. As yet, however, no amino-acid other than glutamine has been reported at positions β139 (H9) (ref. 11).

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Paraffin Synthesis in the Oenocytes of the Desert Locust

OENOCYTES are one of the least understood types of insect cells. Several workers have suggested that the oenocytes produce structural lipoproteins for the formation of the cuticle or the paraffins and waxes which are involved in water-proofing the cuticle[1-4]. Electron microscope studies[5-9], ligation experiments[10,11] and biochemical studies[11-13] suggest that the oenocytes rather than the prothoracic glands synthesize the moulting hormone ecdysone. At present, however, there is no direct and conclusive evidence to support either of these hypotheses.

We have investigated the cytology and function of the oenocytes in the desert locust *Schistocerca gregaria*. The animals used were fifth instar female larvae fed on bran and wheat seedlings and exposed to a day of 12 h (35° C) followed by a night of 12 h (25° C). The instar lasts about 9 days. The oenocytes are found only in the peripheral abdominal fat body in association with ordinary fat body cells and with some small "urate cells". The central fat body surrounding the gut contains only ordinary fat body cells. The extensive smooth endoplasmic reticulum which we have observed in the oenocytes suggests that these cells synthesize lipids. We therefore carried out incorporation studies using ^{14}C-acetate with both types of fat body and with hypodermal cells from the tergum of the first thoracic

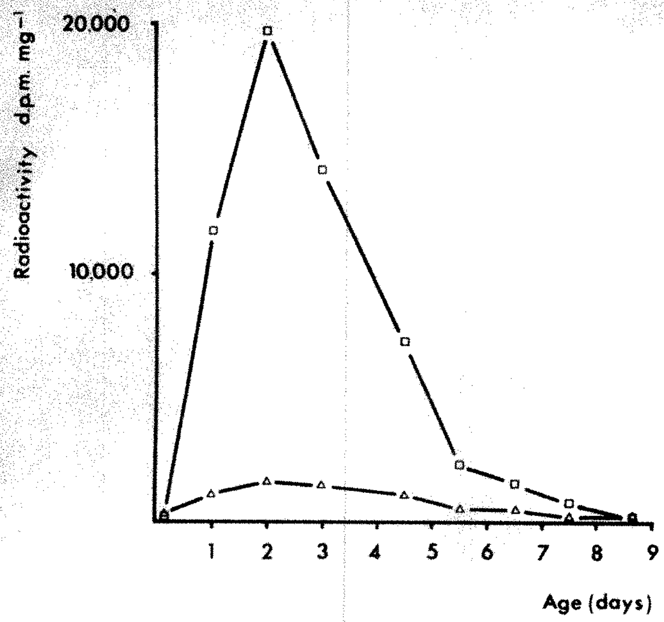

Fig. 1 ^{14}C-labelled lipids from the central yellow fat body during the development of the fifth instar in *Schistocerca gregaria*. Radioactivity (in discharges per minute, d.p.m.) in phospholipids (△) and triglycerides (□) per mg tissue (wet weight). The instar lasts about 9 days.

segment. Dissections and incubations were carried out in a buffered Ringer solution (20 mM PIPES-buffer at *p*H 6.8 containing 125 mM Na^+, 10 mM K^+, 2 mM Ca^{2+}, 2 mM Mg^{2+}, 143 mM Cl^-, and 2.5 mM glucose). In each experiment 100 mg of fat body or 4 terga, freed from other tissues, were incubated for 1 h at 35° C in 2.5 ml. of the Ringer containing 1.6 μCi of uniformly labelled ^{14}C-Na-acetate (0.03 μM). After homogenization the lipids were extracted with chloroform-methanol (2 : 1 v/v)[14], separated by thin-layer chromatography on silica gel G using several solvent systems of differing polarity and identified by comparison with lipid standards. Radioactive spots were located with a plate scanner, removed from the plate and counted by liquid scintillation counting.

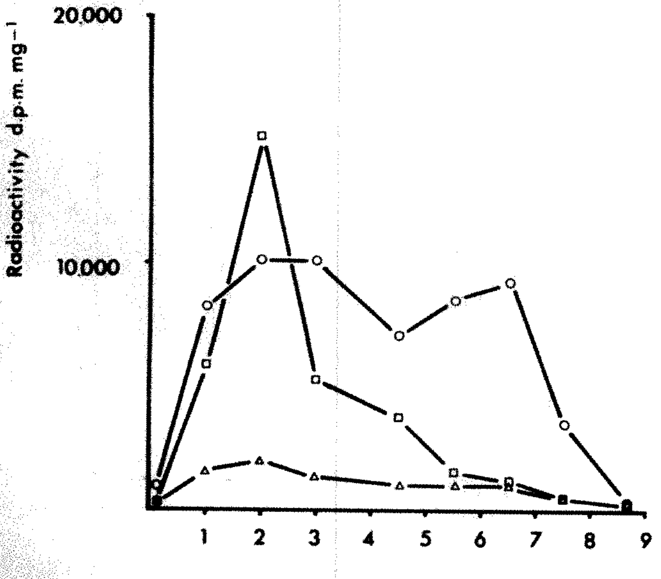

Fig. 2 ^{14}C-labelled lipids from the oenocyte-rich peripheral fat body. Radioactivity (d.p.m.) in phospholipids (△), triglycerides (□) and paraffins (○) per mg tissue (wet weight) determined at various stages of the fifth instar.

Incorporation of the radioactive precursor was followed daily throughout the instar. Figs. 1 to 3 show that both phospholipids and triglycerides were labelled extensively in all three tissues. In both types of fat body the high rates of incorporation into triglycerides confirm previous observations[15] that triglycerides are the principal lipids synthesized by and stored in the fat body of *Schistocerca*. In the oenocyte-rich peripheral fat body, however, there is a third extensively labelled lipid fraction which is absent from the central fat body (Fig. 2). Thin-layer chromatography suggests strongly that these lipids are paraffins because they co-chromatograph in hexane with paraffin standards. Several lipids of unknown nature were also labelled to a small extent. (The data for these small fractions are omitted from Figs 1 to 3.) The hypodermis also synthesizes paraffins (Fig. 3), but the amount is so small that the incorporation of ^{14}C-acetate into paraffins by the entire hypodermis is less than 1% of that simultaneously incorporated by the oenocyte-rich fat body. Thus the synthesis of paraffins must take place primarily in the oenocyte-containing peripheral fat body.

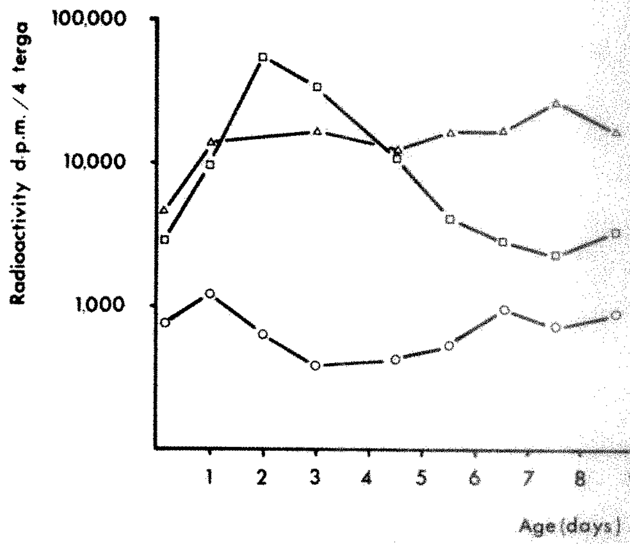

Fig. 3 ^{14}C-labelled lipids from the hypodermis of the tergum of the first thoracic segment. Radioactivity (d.p.m.) in phospholipids (△), triglycerides (□) and paraffins (○) determined at various stages of the fifth instar. Four terga were used in each experiment. Note that the vertical scale is logarithmic by contrast with those of Figs. 1 and 2.

The "paraffin" fraction isolated by thin-layer chromatography was analysed by gas-liquid chromatography on a SE-30 column. Most of the components showed retention times similar to those of the n-alkanes C_{21}–C_{33} with the odd-numbered C_{25}-, C_{27}-, C_{29}- and C_{31}-*n*-alkanes dominating. Infrared spectroscopy also suggests that these lipids are saturated paraffins.

Our cytological and histochemical studies suggest that the oenocytes are responsible for the synthesis of paraffins. This is supported by a cell separation experiment. Oenocyte-rich fat body from 5-day-old larvae was incubated as above and then placed for 10 min in Ringer solution at 35° C containing 0.05% 'Pronase'. Cell separation was facilitated by repeatedly drawing the tissue sample through a pipette. Centrifugation at low speed (3–5*g*) for 1 min left most of the fat body cells in a froth at the surface. Most of the "urate cells" remained in the supernatant. The pellet contained an abundance of oenocytes with a few fat body cells and "urate cells". In the pellet the ratio between the radioactivity in the paraffin fraction and in the triglyceride fraction was 13 : 1 whereas in the intact control tissue the ratio was 2.3 : 1. This enhancement of the paraffin fraction following partial separation of the oenocytes strongly supports the view that these cells are responsible for paraffin synthesis and that triglyceride synthesis is mainly associated with the ordinary fat body cells.

The paraffins synthesized in the oenocytes must be transported to the cuticle where they make up 40–60% (w/w) of the chloroform-soluble cuticle lipids. Indeed, after *in vivo* incorporation of ^{14}C-acetate, paraffins are the most extensively labelled of the cuticular lipids. From the above results, we conclude that in *Schistocerca* larvae a large proportion of the cuticular lipids is synthesized by the oenocytes and that this must be one of the main functions of these cells. The role of oenocytes in ecdysone synthesis, however, remains uncertain. ^{3}H-cholesterol injected into locusts without prothoracic glands is converted into α and β-ecdysone (identified by thin-layer chromatography of their trimethylsilyl derivatives[16]). At present one cannot exclude the possibility that oenocytes may be responsible for this conversion.

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Suppression of the Cyclic Surge of Luteinizing Hormone Secretion and of Ovulation in the Rat by Δ^1-Tetrahydrocannabinol

IN the rat, a surge of gonadotrophin secretion occurs during the afternoon of the day of pro-oestrus[1,2]. This induces the resumption of the first maturation division of the oocyte and changes in the follicular wall that culminate in ovulation—the release of the mature secondary oocyte. The cyclic discharge of ovulating hormone, principally pituitary luteinizing hormone (LH), depends on the secretion of LH-releasing hormone (LRH) by the hypothalamus[3]. LRH secretion, and consequently LH surge and ovulation, can be blocked by several neurodepressive drugs[4].

Δ^1-Tetrahydrocannabinol (also known as Δ^9-tetrahydrocannabinol—the designation Δ^1 corresponds to the nomenclature proposed by Mechoulam[5]) (THC) is the chief psychoactive ingredient of marihuana[5]. It acts on the central nervous system, causing changes in the EEG[6] and in the metabolism of 5-hydroxytryptamine and catecholamine[7–10]. The drug also activates the pituitary-adrenal axis[11].

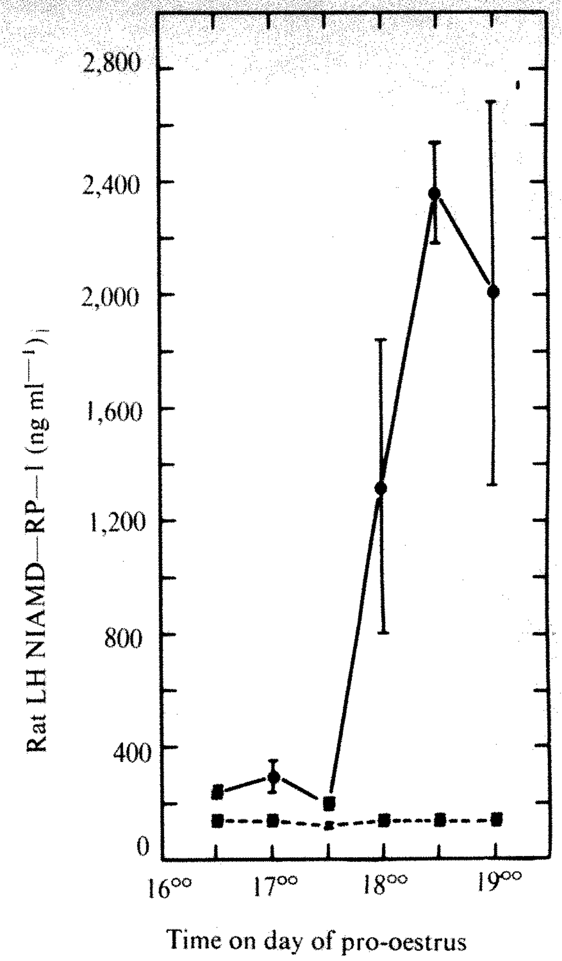

Fig. 1 Effect of Δ^1-tetrahydrocannabinol (THC) on the concentration of serum LH of pro-oestrous rats. Intraperitoneal injections of THC (1.0 mg per rat each) were given at 1400 and 1600. Control animals received two injections of the vehicle. Vertical brackets indicate ± s.e.m. ——, Control rats; - - - -, rats treated with THC.

We report here an effect of THC on the serum concentration of LH and on ovulation in the rat. Three-month-old Wistar rats weighing approximately 200 g were kept in quarters illuminated between 0500 and 1900. All had shown at least two normal 4-d-cycles, as determined by daily vaginal smears, immediately before the experiment. THC (2 mg per rat, or approximately 10 mg kg^{-1}), divided into two equal doses, was injected intraperitoneally into one group of twenty-four rats at 1400 and 1600 on the day of pro-oestrus. A control group received injections of the vehicle (a Tween-propyleneglycol–water mixture). Between 1630 and 1900, blood samples were drawn by cardiac puncture under light ether anaesthesia from four animals in each group at intervals of 30 min. No rat was bled more than once. The concentration of LH in the serum collected from these samples was determined by radioimmunoassay[12]. As shown in Fig. 1, treatment with THC prevented the characteristic afternoon rise in the concentration of serum LH which occurs in the control group.

In another experiment, rats treated with THC on the day of pro-oestrus at the times and dose-levels specified in Table 1, and vehicle-injected controls, were killed the following morning and the oviducts were examined for the presence of shed ova. If ovulation had not occurred, the oocytes retained in enlarged follicles were aspirated and examined microscopically; oocytes devoid of a germinal vesicle and nucleolus were classed as mature.

THC (total dose 2 mg per rat) effectively blocked ovulation (Table 1). In five of the twenty animals in which ovulation was blocked by THC, however, the oocytes entrapped in the follicles were found to have matured. At lower doses (0.4–0.8 mg per rat total dose), the concentration of serum LH was significantly lowered (50% reduction; $P<0.05$, $n=41$), but not sufficiently

Table 1 Suppression of Ovulation by Δ¹-Tetrahydrocannabinol (THC)

Treatment			Ovulation	
THC (mg per rat)*	Nembutal (mg kg⁻¹ body weight)†	LH (μg per rat)‡	No. of rats / No. treated	Mean No. ova per ovulating rat
— (Vehicle only)	—	—	26/27	10.4
0.4–0.8	—	—	7/8	10
2.0	—	—	1/21	1
—	30	—	0/6	—
—	30	2.5	6/7	12.0
2.0	—	2.5	4/10	8.3

* Divided into two equal doses given intraperitoneally (i.p.) at 1400 and 1600 on the day of pro-oestrus. Oviducts inspected at 0900 the following day.

† Given intraperitoneally at 1400 on day of pro-oestrus.

‡ Given intraperitoneally at 1630 on day of pro-oestrus.

to prevent ovulation (Table 1). All THC-blocked rats ovulated 24 h after the normal time. A similar delay in ovulation occurs in rats after treatment with pentobarbital sodium (Nembutal)[4].

Treatment with LH, at a dose sufficient to cause ovulation in Nembutal-blocked rats (2.5 μg per rat), caused only 40% of the animals treated with THC to ovulate (Table 1). So a direct effect of THC on the ovary, in addition to its suppressive effect on secretion of LH, cannot be excluded. Accumulation of THC in corpora lutea after intravenous injection of the drug into mice has been reported[13]. Indomethacin, an inhibitor of prostaglandin synthesis, blocks the ovulatory action of LH at the ovarian level[14]. It remains to be seen whether the two drugs act on the ovary in a similar manner[15].

The cyclic surge of LH is particularly sensitive to agents affecting the central nervous system. It cannot be concluded from our experiments that THC has a similar inhibitory action on the steady, "tonic" secretion of LH in either sex. Neither do we know whether the anti-gonadotrophin and anti-ovulatory actions of THC observed are caused by the drug itself or by one of its metabolites[16]. Finally, it should be noted that the doses used in these experiments were high (2 mg per rat) compared with the estimated intake by human drug users (probably <1 mg per man), and the route of administration differed.

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Uterine Activity following Intravenous Administration of Oxytocin to the Foetal Sheep

CONCENTRATIONS of oxytocin in umbilical cord blood are high in the newborn human[1] and in several species the foetal pituitary contains large amounts of oxytocin and neural lobe hormone carrier proteins, the neurophysins[2,3]. If the foetal posterior pituitary is to play a part in labour, however, it must not only secrete oxytocin but this oxytocin must pass from the foetal vasculature to the myometrial cell. No observations have been reported regarding the ability of oxytocin to produce uterine contractions when administered from the foetal side of the placenta.

In five pregnant ewes of gestational age 135–139 days catheters were placed in the foetal dorsal aorta and inferior vena cava and a branch of the maternal uterine vein[4]. In one animal with a twin pregnancy, intravascular catheters were placed in each foetus. A saline-filled balloon was placed in the uterus at the same time to monitor uterine pressure changes. Two days were allowed to elapse before any experiment was performed. Progesterone and oestrogen were measured using antisera donated by Dr G. Abraham. Plasma was extracted with ether and measured by a modification of the method of Abraham *et al.*[5], but without chromatographic separation. By this method the recovery for progesterone was 90% and 93% for total oestrogens. Plasma cortisol was measured by the competitive protein binding method of Murphy[6], with a recovery of 87%. No correction has been made for recovery in the values reported.

All intravascular injections were given in 1.0 ml. of physiological saline (0.9% NaCl w/v) infused over 10 s. At the beginning of each experiment a control injection of saline was given into the foetal venous catheter and the uterine pressure observed for a period of 30 min. This was followed by a control injection into the maternal uterine vein catheter. After a further 30 min 100 mU 'Syntocinon' (Sandoz) was administered through the foetal catheter. An hour later the same

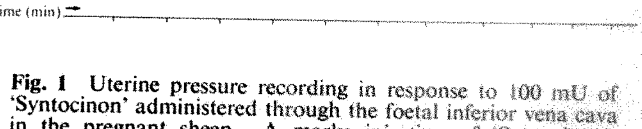

Fig. 1 Uterine pressure recording in response to 100 mU of 'Syntocinon' administered through the foetal inferior vena cava in the pregnant sheep. A marks injection of 'Syntocinon'. Sheep No. 67 on day 141. Foetal cortisol was 8.1 μg per 100 ml., rising to 12.5 μg per 100 ml. on the following day. Maternal oestrogens were 0 pg ml.$^{-1}$ rising to 450 pg ml.$^{-1}$ on the following day.

Table 1 Effect of Maternal and Foetal Administration of Syntocinon on Uterine Activity in Sheep of Known Gestational Age

Animal No.	Route of administration and dose; single injection of 100 mU syntocinon	Increase in uterine pressure	Latency	Foetal corticosteroids μg 100 ml.$^{-1}$	Maternal progesterone ng ml.$^{-1}$	Maternal total oestrogens pg ml.$^{-1}$
161 (139)*	Foetal	–		3.6	6.1	0
(139)	Maternal	++	30 s			
(140)	Foetal	–		5.3	7.5	0
(140)	Maternal	++	60 s			
(141)	Foetal	++	2.5 min	7.2	4.7	0
(141)	Maternal	++	30 s			
159 (139)	Foetal	–		3.7	19.8	264
(139)	Maternal	+	2 min			
(140)	Foetal	+	4 min	5.7	17.7	238
(140)	Maternal	+	2 min			
(141)					10.8	370
Birth (142)					1.7	1,132

Data are given for plasma concentrations of maternal oestrogen and progesterone and foetal corticosteroids.
* Gestational age.

dose of 'Syntocinon' was given to the mother. The four animals with single foetuses were tested again the following day and one of these (No. 161, see Table 1) on the third day. Two animals delivered live foetuses within 24 h of the last experiment to test the response to oxytocin. In the other three, live foetuses were delivered by caesarean section after the last experiment. The ewe with the twin pregnancy was only investigated on one occasion and immediately afterwards the lambs were delivered by caesarean section.

Infusion of saline into the foetal or maternal catheter did not produce contractions on any occasion. In two animals with single foetuses, foetal injection of oxytocin produced an increase in uterine pressure at each administration. In the other two animals with single foetuses shown in Table 1, the first injection of oxytocin on the foetal side was without effect. Foetal administration of oxytocin did produce contractions on day 140 in sheep 159 and day 141 in sheep 161. No effect was obtained on day 140 in sheep 161. In each case the appearance of a uterine response to foetally injected oxytocin was associated with a lower maternal jugular plasma progesterone concentration and a higher foetal plasma cortisol than on the occasion when no response was obtained. In the twin pregnancy injection of 'Syntocinon' into either foetal catheter produced contractions. During the 3 h of the experiment, plasma cortisol in one foetus rose from 6.3 to 10.5 μg per 100 ml. and in the other from 3.6 to 5.0 μg per 100 ml. These values suggest that parturition was imminent[7].

The type of contraction pattern elicited by 100 mU oxytocin injected into the foetus is shown in Fig. 1. There was always a general rise in uterine tone on which were superimposed several small contractions. The latency of the response was 2.5–4 min. Large rhythmic contractions were never seen after the foetal administration of oxytocin but the contraction pattern may be different closer to the time of normal delivery.

The administration of oxytocin to the mother invariably resulted in increased uterine activity (Table 1). Although the contraction pattern was largely similar to that observed after a foetal injection, the following differences were found. After the maternal injection there was a shorter latency to the onset of the contractions; the duration of increased uterine pressure was longer; the increase in pressure was greater and there was more tendency to establish rhythmic contractions. In two animals an increased response to maternal administration of oxytocin was noted on the second occasion the animal was tested.

These results show that 100 mU oxytocin (200 ng) injected into the foetal circulation can stimulate the myometrium near term in the sheep and that the latency of this response is considerably longer than when the same amount of hormone is given directly into the maternal circulation, despite the very much greater maternal plasma volume. The longer latency of the response to the hormone when given intravenously to the foetus suggests that placental transfer must be slow. Further work is necessary to elucidate the pathway by which oxytocin passes from the foetal blood to cause contraction of the myometrium.

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Chimaeras Obtained by Aggregation of Mouse Eggs with Rat Eggs

TECHNIQUES have been developed which enable mouse eggs differing in age and stage of development to aggregate and form large but apparently morphologically normal blastocysts capable of egg cylinder formation at least[1,2]. Such chronological regulation has also been demonstrated for the mouse blastocyst by transfer of an isolated 4.5-day inner cell mass to a 3.5-day blastocyst with the subsequent formation of a normal chimaera[3]. These systems show that chimaeras derived from two components with the same pattern of differentiation, but with a temporal discrepancy experimentally imposed, can develop normally. This suggests that rat and mouse eggs, in which the pattern of differentiation is similar but on a different time scale, might develop successfully when aggregated together. This hypothesis was supported by a pilot study[4] which has been extended and is reported here.

Eggs were collected from Q strain mice and Wistar rats

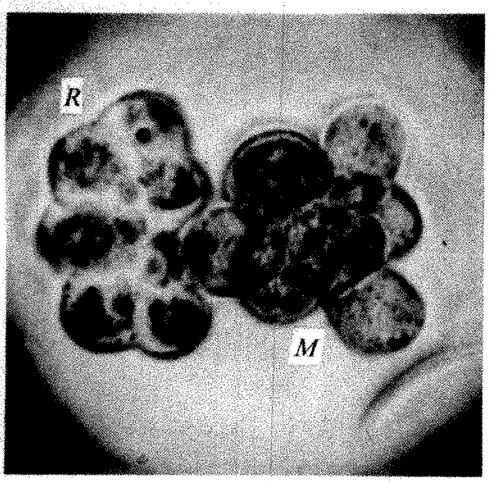

Fig. 1 An eight-cell mouse egg (*M*) conjoined with an eight-cell rat egg (*R*).

and cultured in Whittingham's medium[5]. They were prepared for aggregation using routine techniques[1].

Of fifty-nine eight-cell rat eggs paired with mouse eggs at four-cell, eight-cell or early morula stages, thirty-six (61%) aggregated together (Table 1). Of these, sixteen out of fifty-nine (27%) formed blastocysts which appeared morphologically normal. Although total incorporation of both components occurred in ten cases a few cells were excluded from the other six chimaeric blastocysts. The exclusion of some cells is not, however, uncommon in such experimental studies[2]. The remaining twenty (34%) chimaeric aggregates failed to develop a blastocyst cavity. Whether this was the result of manipulative procedures, culture conditions or a factor inherent in the eggs themselves is not known.

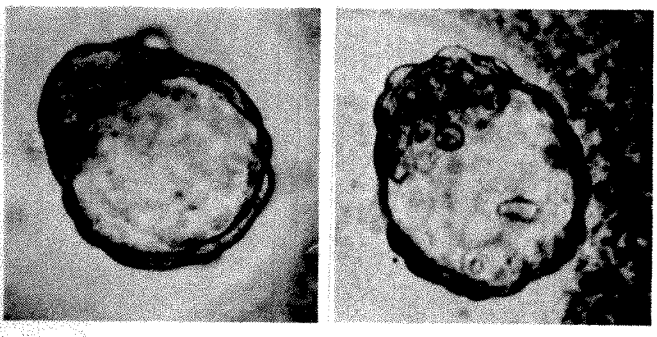

Fig. 2 The blastocysts developed from pairs of mouse and rat eggs similar to those shown in Fig. 1.

Table 1 Development of Conjoined Pairs of Rat and Mouse Eggs

Stages* conjoined	Degree of development No aggregation	Aggregation but no blastocyst cavity formed	Blastocyst formation
8×4	1	4	1
8×8	20	16	13
8×EM	2	—	2
Total	23 (39%)	20 (34%)	16 (27%)†

* The first named egg of each pair is the rat egg. † This compares with 42% successful blastocyst formation from asynchronous mouse/mouse aggregates[1]. EM, Early morula.

This study shows that the difference in intrinsic rate of development between rat and mouse does not form an obstacle to their aggregation and cooperation in subsequent blastocyst formation. Furthermore, apparently normal blastocysts can be produced when the pairs of eggs conjoined are of differing cleavage stages (Table 1). The remarkably flexible developmental potential of the rodent egg, thus demonstrated, offers an opportunity of following the interaction of genotypically different cell populations during differentiation and, further, the possibility of elucidating aspects of foetal/maternal interactions such as implantation.

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Impairment of Latent Learning in the Rat by a Marihuana Component

NUMEROUS studies in both humans[1-3] and animals[4] indicate that marihuana and its chief psychoactive constituent, Δ^9-tetrahydrocannabinol (Δ^9-THC), may have disruptive effects on short term memory. It has been suggested, however, that the memory-disrupting qualities of marihuana in humans may actually be incidental to its effect on normal attentional processes[5]. Furthermore, interpretation of animal investigations in terms of drug-induced memory disruption may be difficult as Δ^9-THC may alter appetitive motivation[6]. So it is unclear whether performance deficits are a consequence of impaired memory, attention, or motivation.

One way to circumvent possible motivational problems and at the same time study the effects of Δ^9-THC on attention is to use an experimental paradigm which takes into account both of these factors. In a latent learning paradigm, rats can be pre-exposed to a distinctive environment such as a maze for some time with no food and can be allowed to explore freely. Subjects can then be deprived during an acquisition phase with food introduced to determine whether they benefited from their exploratory experience in terms of some measure such as reduced error scores in comparison to non-exposed controls. If Δ^9-THC interferes with the ability to attend to significant environmental cues, animals treated with the drug during free exploration may not show any benefit from their familiarization period when tested during an acquisition phase in a drug-free state.

The subjects were thirty-two naive male hooded rats 100–110 d old at the start of the experiment. The apparatus consisted of a seven-unit multiple Y maze with interchangeable steel units. A grey wooden box with hinged top and guillotine door served as a goal box. Individual Y units were arranged so that the sequence of correct choices was LLRRLRL.

The latent learning procedure which was used resembles the type II design identified by MacCorquodale and Meehl[7] which consists of free exploration followed by reward. After a brief period of handling, all rats were habituated for 2 d to a single Y unit located on the floor for 15 min d^{-1}. After habituation, subjects were randomly assigned

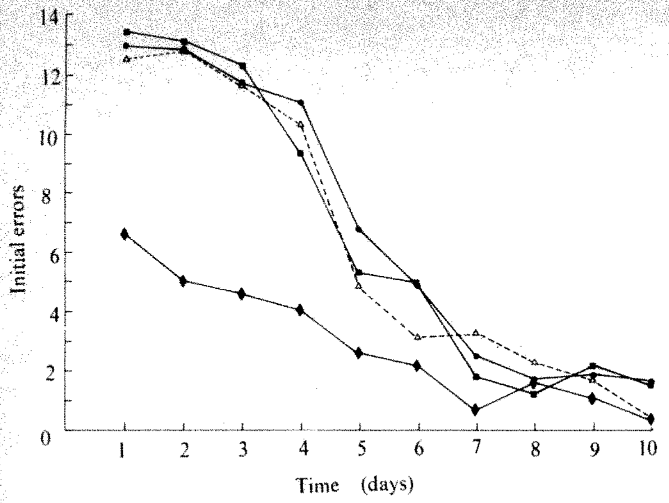

Fig. 1 Effects of Δ^9-THC on the mean number of initial errors over 10 acquisition days. ●, CON-D; ■, LAT-D; △, CON-ND; ◆, LAT-ND.

to one of four groups, latent learning-drug (LAT-D); latent learning-no drug (LAT-ND); control drug (CON-D); control-no drug (CON-ND). Subjects in the two latent learning groups were exposed to the multiple Y maze for 15 min d^{-1} for 3 consecutive days. Although the goal box was not baited on exposure days, the small glass tray which was to be used as a food receptacle was present. The two control groups received continued exposure to the single Y unit for the 3-d period with goal box conditions identical to those experienced in the multiple Y maze. Thirty minutes before exposure on each of the 3 d the subjects were dosed intraperitoneally with either vehicle or 3 mg kg^{-1} Δ^9-THC in 1% Tween-80-water.

The choice of a dose of 3 mg kg^{-1} was based on pilot observations which indicated that although this dose was maximally effective in blocking latent learning, no reduction in cul entries occurred. The number of culs entered during exploration is inversely related to the number of errors made during testing[8]. Doses higher than 3 mg kg^{-1} reduced the number of culs entered during exploration. After each exposure subjects were returned to their home cage and allowed access to the food pellets which were to be used in the acquisition phase. During the exploration phase subjects were reduced to 85% body weight. Although the procedure of depriving the animals during exploration may not be desirable because of a possible interaction between drug treatment and deprivation condition, it was used because a pilot study indicated that two 24 h deprivation periods immediately after the last day of exposure were not sufficient to motivate the rats to eat in the goal box.

On the day after completion of exploration, each subject was given four rewarded placements in the goal box, receiving two 250 mg Noyes pellets on each trial. Acquisition was begun on the next day. All subjects received four trials a day for 10 d in the multiple Y maze with intervals between trials of 1 min. Two types of errors were recorded: initial errors, which consisted of a subject making an incorrect turn at a choice point; and retracing errors, which consisted of a subject making the correct turn at the choice point and then retracing and committing an error.

A 2 (latent against control) by 2 (drug against no drug) by 10 (acquisition d) analysis of variance was performed on the mean number of initial errors made during acquisition. These means are plotted across acquisition days in Fig. 1. The most relevant features of this analysis were the highly significant interaction between the latent-control effect with days ($F=2.90$, $d.f.=9{,}252$, $P<0.005$) and drug-no drug effect with days ($F=2.67$, $d.f.=9{,}252$, $P<0.01$). The triple interaction also reached significance ($F=2.85$, $d.f.=9{,}252$, $P<0.005$). Comparisons of individual cell means revealed that the group receiving vehicle during maze exposure made significantly fewer errors over the first 5 days when compared with group CON-ND ($P<0.01$). The LAT-D group did not differ from its respective control group, indicating that THC nullified the latent learning effect. The LAT-D, CON-D, and CON-ND groups also did not differ from one another. The incidence of retracing errors was quite low and did not differ among the various groups. A t test indicated that the mean numbers of cul entries made by subjects in the LAT-D and LAT-ND groups were not different, although the number of culs entered each day decreased over the 3 d exposure period.

Although no final conclusions can be drawn on the basis of a single study, our results suggest that rats exposed to the maze under the influence of THC may have attended to fewer of its salient features and therefore learned less about the correct path to the goal. It is also possible that, under THC, erroneous response patterns were established during exploration and these carried over to acquisition. If THC blocks habituation as Brown[9] has contended, the probability of entering previously explored but incorrect blinds would remain high. In a design of this type one cannot, however, rule out with certainty that the lack of a latent learning effect in group LAT-D could be a result of dissociation brought about by the change in drug state[10] from exploration to acquisition.

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Electrical Method for Controlling Pain

An instrument capable of producing effective dental anaesthesia has recently attracted some publicity in Britain, although no reports have yet been published. Briefly, a controlled constant current is provided by a 22 V battery, the positive lead being connected to a dental drill, insulated at the handle, and the negative lead attached by a metal clip to the ear of the patient. Current flow is controlled to give an output ranging from 0 to 60 μA as registered on a meter. The instrument, which is of Russian design and marketed in West Germany, is both compact and portable. Preliminary studies on a number of patients suggest that anaesthesia is effective with constant

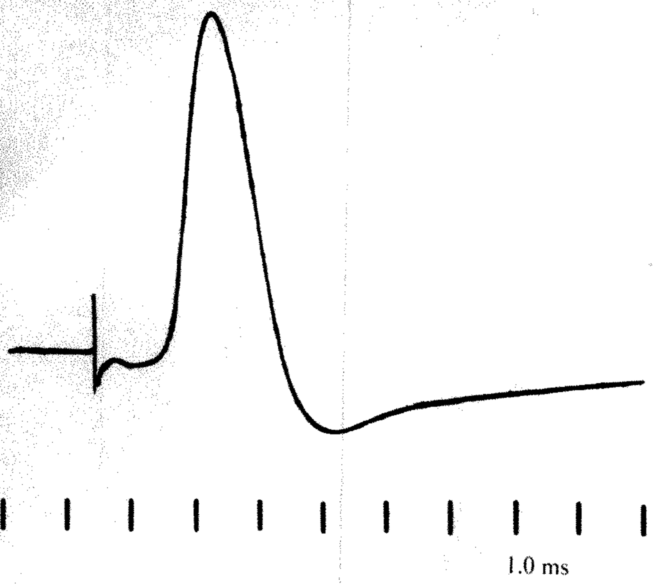

Fig. 1 Compound action potential of frog sciatic nerve. Maximal response with single stimulus 2.5 V, 0.01 ms. Impulses are conducted in all the fibres of the nerve trunk.

currents ranging between 45 and 60 μA. In several drilling procedures that would have required injections of local anaesthetic in normal practice, the patients did not feel any pain.

There are several ways of inducing blocking of nerve impulses, local anaesthetics, cold, heat and pressure being the more usual agents. For example, below a certain temperature the propagation of the excitatory process is abolished, and various narcotics such as procaine diminish conductivity of the nerve fibres.

To demonstrate the method of anodal blocking, the frog sciatic nerve was stimulated at its distal end by means of a square wave pulse generator and the nerve action potentials, recorded from a proximal pair of electrodes, were displayed on an oscilloscope. The strength of the stimulus was arranged to produce a maximal response in order to excite all the individual fibres (Fig. 1). When the output leads of the instrument were connected to a portion of the nerve between stimulating and recording electrodes, changes were induced by the flow of anodal current, as shown in Fig. 2. As the strength of the current was increased, there was a progressive reduction in the amplitude of the action potentials with prolongation of conduction times until complete blockade was achieved. The depression was maintained during current flow and persisted for a few seconds after the current was switched off. Full recovery of the action potential was observed after about 10–15 s. This procedure could be repeated many times without any obvious signs of fatigue in the nerve or changes in the form of the records.

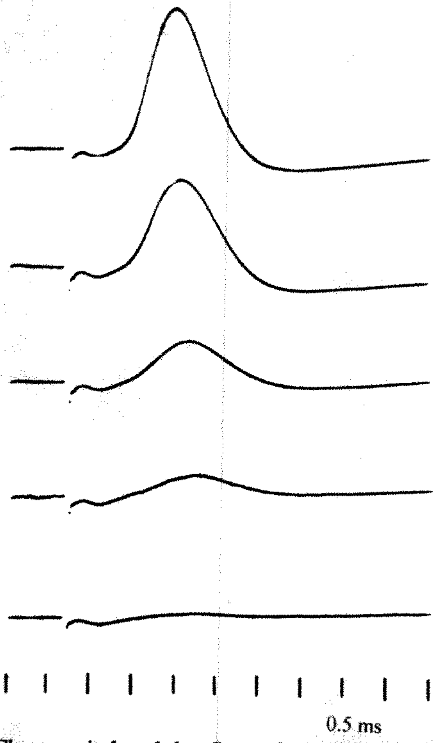

Fig. 2 Changes induced by flow of anodal current. Note progressive reduction in amplitude until action potential is abolished. Conduction of all nerve impulses is blocked.

The potentials induced in a nerve membrane by an electrical stimulus are well known: the local state of polarization is disturbed and the membrane becomes freely permeable to ions. The depolarized region of the nerve fibre initiates a local flow of current which extends into the adjacent resting membrane. This in turn becomes depolarized and a nerve impulse is propagated along the fibre. The effect of an applied anodal current is to oppose these changes either by restoring the polarized state or by producing hyperpolarization of the nerve membrane. The latter would account for the observed period of after-depression.

Although the concept of anodal blocking of nerve impulses has been evident for many years, its application to the clinical relief of pain is quite new. The apparent success of the method in dental anaesthesia is at least based on sound physiological principles and it may become the method of choice for treatment of children and for very nervous patients or for those with a high degree of sensitivity for pain. It is also possible, with suitable modifications, that the same principle could be applied to other regions of the body, for example, in the treatment of fractures and emergency conditions that usually require the administration of a general anaesthetic.

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Potato Blight Teratogen

LAURENCE[1], in his criticism of Renwick's inference that potato blight is the principal cause of spina bifida and anencephaly, states: "In any case it is likely that one is dealing not with a single teratogen malformation but with one which is multifactorial". This is difficult to parse, but, as multifactorial is now a fashionable word, and as it is important to try and reduce the emotion, to which Laurence objects so emotionally, this word, at least, might be eliminated from meaningful discourse.

All conditions are, to some extent, variable and, to some extent, this variability is reduced in subgroups defined in either genetical or environmental terms. To say "X disease is multifactorial, or polygenic, or complex, or part-genetic" (to use some peculiarly confusing neologisms) is a statement of the same order of informativeness as "Moon dust is made of chemicals" or "mice are made of atoms". It could not be otherwise.

The relationship between multifactorial inheritance and environmental influence is such that multifactorially determined predispositions, of any strength, can be formally irrelevant to the opportunities for environmental modification. In principle there seems no difficulty in eventually breeding a race of men who could smoke with impunity; meanwhile a 95% reduction in the incidence of lung cancer could be achieved in a generation if smokers gave up the habit. There is no incongruity between Renwick's claim of 95% reduction and Laurence's claim of genetic or other factors. Even if some genetic counsellors have presumed to add spina bifida to those disorders which carry a genetic risk, although—so far as I know—none have given genetic advice to relatives of victims of smoking, this is no reason to suppose spina bifida to be less optional than lung cancer. Nor is there any problem of only one of a pair of twins being affected. Only a minority of smokers develop lung cancer. Nor is there any incongruity in such reckless hormonal gauntlets as *in vivo* pregnancy diagnosis being a substantial contributor to spina bifida.

In the data of Laurence *et al.*[2], cited by Laurence[3] (also personal communication to Laurence, from Laurence, quoted by Laurence), a most detailed and bias-resistant study gave a maximum likelihood estimate of the proportion of spina bifida caused by hormonal trauma, in those exposed to this trauma, of (323-271)/323 or about 15%.

If it is true that about 7% of women, with their general practitioners, have a sufficient joint irresponsibility to the unborn to diagnose pregnancy in this way, then the best estimate of this "factor" is that it is related to almost 1% of cases, or more than one a month. This is, of course, a very vague estimate—the true figure may be anything from none a year, as Laurence asserts, to one or more a week. This is significant in one sense and not in another. As many authors follow Laurence in confusing statistical significance with biological significance, this is another word which might usefully be avoided unless defined when used.

Finally, if I follow the anti-potato letter, Laurence regrets that the statistics are being disturbed by women who get pregnant and fail to eat potatoes. In view of the extensive, considerable, and consistent data on the recurrence risks of these disorders it is difficult to see why a trial should be controlled. It is only necessary for a few hundred women to fail to produce a second affected child on a potato-free diet, or for a few women to have this misfortune while dieting, to clarify this issue, if, indeed, the chemists and experimental teratologists do not get there first.

Fortunately the consequences of less than half the population not eating potatoes for less than a fiftieth of their lives will hardly have any very serious nutritional or economic consequences and it is difficult to see anything but good coming from the uncontrolled non-eating of potatoes in early pregnancy.

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Associations between the Incidence of Neurological Malformations and Potato Blight Outbreaks over 50 Years in Ireland

RENWICK[1] has suggested that anencephalus and spina bifida in man are possibly caused by a specific but so far unidentified teratogen in potato tubers infected by the blight fungus, *Phytophthora infestans*.

The consumption of potatoes per person in Ireland is high and infestation of the annual crop by the blight fungus is also prevalent[2]. Of all the areas so far studied on a community basis Belfast and Dublin have the highest frequencies of anencephalus[3]. A similar geographical variation probably exists with regard to the incidence of spina bifida. In 1957, McKay[4] presented a retrospect of 50 yr (1907–1956 inclusive) of outbreaks of potato blight in the Irish Republic. Estimates of the number of outbreaks recorded by the end of June during each year were based on specimens collected by potato inspectors and other local officers, and blight infestation was verified by microscopic examination. We have just completed some studies of epidemics of anencephalus and spina bifida in Ireland[5,6] based on data reported from the three main Dublin maternity hospitals for 1900 to 1965.

Table 1 Analysis of Variance of the Association Between the Annual Incidence of Anencephalus in Births reported from the Rotunda Hospital, Dublin, 1908-1957, and the Frequency of Blight Outbreaks in the Irish Republic during the Previous Year after allowing for Secular Trend.

Source of variation	Degrees of freedom	Sums of squares	Mean square	*f* ratio value
Linear trend	2	312.70	156.35	—
Linear trend and blight	3	313.05	104.35	—
Extra attributable to blight	1	0.35	0.35	Not significant
Residual	47	38.55	0.85	—
Total	50	351.60		

$y = 1.075\ (\pm 0.347) + 0.044\ (\pm 0.009) \times \text{time} - 0.0025\ (\pm 0.004) \times$ blight frequency.

We present here some findings of a multivariate analysis correlating secular trends in the annual incidence rates of neurological malformations observed at the largest Dublin maternity unit, the Rotunda Hospital, and McKay's data on potato blight outbreaks.

We found that the annual malformation data can be adequately represented by a linear regression model; there has been a statistically significant linear increase in the incidence of anencephalus ($f2,48 = 192.94$; $P < 0.001$) and of spina bifida ($f2,48 = 73.18$; $P < 0.001$) over the 50 yr. We therefore tested the null hypothesis that there was no association between the annual incidence of anencephalus and the frequency of blight outbreaks recorded by the end of June of the previous year after allowing for the secular trend. A similar null hypothesis was examined with respect to spina bifida. Tables 1 and 2 show the fit of the model

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2$$

to the anencephalic data and the spina bifida data separately, where y = estimated annual malformation incidence, x_1 = time, x_2 = number of blight outbreaks recorded by the end of June of the previous year, β_0 = constant and β_1, β_2 = estimated regression coefficients with standard errors in brackets.

Both analyses of variance show that the extra variation accounted for by taking into consideration a knowledge of the frequency of blight outbreaks is very small and does not significantly affect estimation of the incidence of either anencephalus or spina bifida based on information relating to secular trend alone. The regression coefficients ($\beta_2 s$) associated with the blight factor are not significant.

Our findings must be interpreted in the light of certain

Table 2 Analysis of Variance – Spina Bifida

Source of variation	Degrees of freedom	Sums of squares	Mean square	*f* ratio value
Linear trend	2	241.52	120.76	—
Linear trend and blight	3	242.66	80.89	—
Extra attributable to blight	1	1.14	1.14	Not significant
Residual	47	78.07	1.66	
Total	50	320.73		

$y = 1.542\ (\pm 0.493) + 0.022\ (\pm 0.013) \times \text{time} - 0.0045\ (\pm 0.005) \times$ blight frequency.

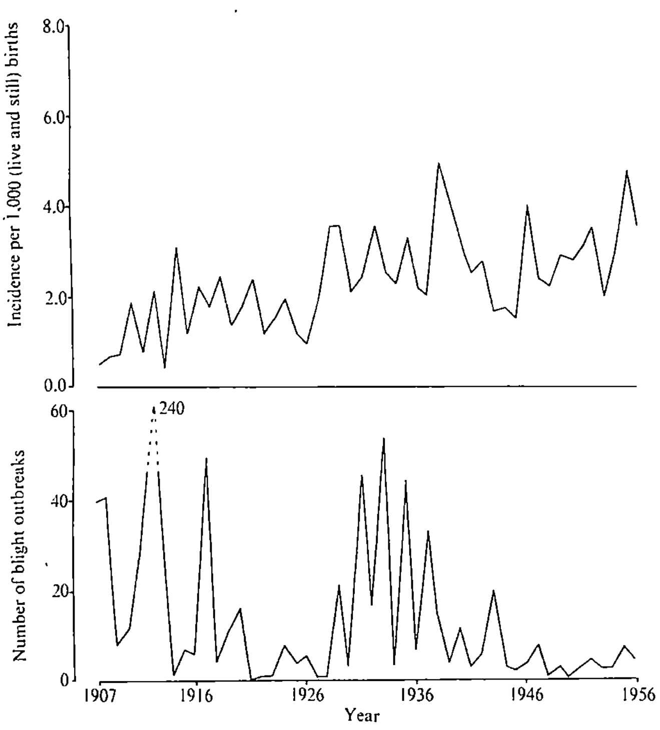

Fig. 1 Annual incidence of anencephalus per 1,000 (live and still) births reported from the Rotunda Hospital, Dublin (upper graph) and the total number of potato blight outbreaks recorded by the end of June of each year in the Irish Republic (lower graph) between 1907 and 1956.

limitations in the two sets of data correlated. Although the malformation data were for hospitals and not the community, as detailed elsewhere[6] we believe that the secular trends observed are genuine and not an artefact. By using McKay's data to test Renwick's hypothesis we assume that the number of potato blight outbreaks is correlated with the proportion of blighted tubers marketed during the ensuing months, which in turn is associated with the amount of such diseased vegetables consumed by expectant mothers in Dublin. No valid agricultural or other data are available adequately to examine this sequence of events.

Another criticism, namely that McKay's figures relate to the whole of the Irish Republic and not to Dublin only, probably is not too important as a substantial proportion of this country's annual harvest is eaten by the 20% of the total population resident in the capital city. With these provisos, however, our analysis provides no evidence of any linear correlation between the incidence of anencephalus or the incidence of spina bifida and the frequency of blight outbreaks in the previous year. The Irish data thus appear to fit less well with the blight hypothesis than, for example, the further data for Scotland[7] and England and Wales[8] which Renwick has published. Whether this is because of the high prevalence of blight almost every year in Ireland—resulting, for example, in an above average number of outbreaks which have little additional effect on a cycle of human development affected by very small amounts of a teratogen—or for other reasons is not known.

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Oxygen as a New Factor controlling Reproductive Growth

IT is well recognized from short term experiments that the photosynthetic and vegetative growth rates of plants with high rates of photorespiration, the so-called C_3 or inefficient plants, are elevated 50 to 100% by sub-atmospheric concentrations of O_2, while those of plants with low rates of photorespiration, the so-called C_4 or efficient plants, is essentially unaffected[1,2]. The long term effect of O_2 concentrations on vegetative and reproductive growth of either C_3 or C_4 plants has not, however, been reported.

We confirm the observation that vegetative growth in soybean (C_3) and sorghum (C_4) is increased by exposure to sub-atmospheric O_2 concentration throughout most of the growth cycle. A previously unrecognized role of O_2, however, which is uniquely associated with reproductive growth, occurs in both C_3 and C_4 plants with greater than the 15% of O_2 required for normal seed development. This observation provides a new approach for elucidation of factors controlling reproductive development and translocation. New O_2 reactions may be involved, and the results may be of significance regarding evolution and ecological distribution.

Table 1 Effect of Sub-atmospheric Concentrations of O_2 on Wye Soybean Growth

	% O_2			
	5	10	15	21
Dry wt (g)				
Leaves	44.2	20.7	8.2	9.2
Stems	25.5	14.4	3.6	5.0
Pods	7.6	12.9	5.8	8.8
Seeds	0	1.1	12.8	21.5
Roots	18.3	14.8	3.4	2.9
No. of fully developed pods	0	11	64	105
No. of undeveloped pods	804	221	35	6
No. of developed seeds	0	12	125	235

Measured at 83 d of age and exposed to the gas phase indicated for 14–83 d. Each datum is the average of two plants grown in separate chambers. Plants were grown with their aerial portion contained in chambers of 'Lucite' acrylic resin of 145 l capacity with urethane foam gaskets around the stem entrance and purged with air or a synthetic gas mixture at 15 l min^{-1}, whilst the root was exposed to air. CO_2 and O_2 contents of the chambers were adjusted to provide 0.03% CO_2 and the desired O_2 concentration and analysed by gas chromatography. Gas mixtures were prepared by dilution of air with N_2 and addition of CO_2. The temperature inside the chambers was 30° C by day and 21° C by night; light intensity 1.47 cd cm^{-2} (4,300 ft. c); day length 12 h. Plants were grown in 18 cm pots containing a 50/50 mixture of 'Jiffy Mix' and sand and were watered daily with Hoagland's nutrient solution.

Results of an experiment in which the aerial portions of soybeans were exposed to 5, 10, 15, or 21% O_2 from 14–83-d plants are recorded in Table 1. The final dry weight of the aerial portion of the plant was increased 74%, by 5% O_2 as against 21% whilst a greater increase (631%) was found for the root. The most striking effects of O_2 concentration were on the morphology and reproductive character of the plants (Figs 1 and 2). There is an increase in lateral development, diameter of stems, and leaf thickness. Some delay in senescence occurs, while the time of flowering is essentially unchanged. The number of fully developed pods and seeds is decreased by all sub-atmospheric levels of O_2 so that at the time of normal maturation there are neither completely developed pods nor seeds for plants exposed to 5% O_2, and only 53% as many

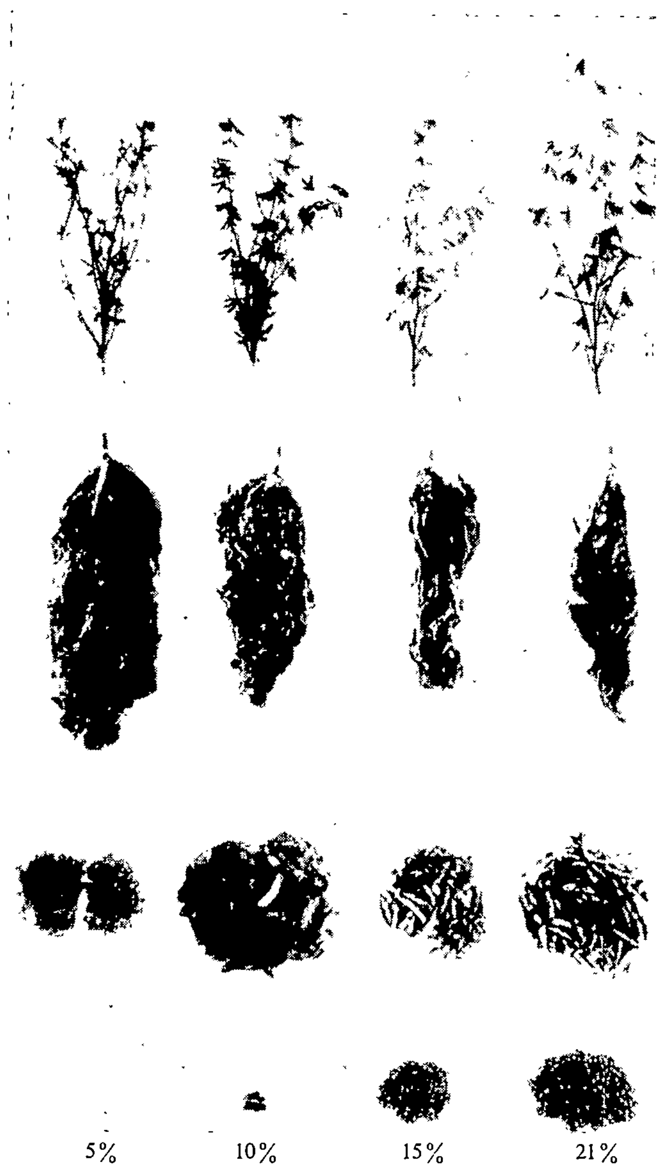

Fig. 1 The effect of sub-atmospheric concentrations of O_2 on growth and development of various plant parts of soybeans; *a*, aerial-portion-defoliated; *b*, roots; *c*. pods; *d*, seeds. (See Table 1 for procedure.)

developed seeds in 15% O_2. On the other hand, the total number of pods is increased by low levels of O_2. With 5% O_2 over 800 pods per plant are produced, which is equivalent to 7.24 times the number obtained with plants grown in air (21% O_2). Similar results have been obtained in four separate experiments with levels as low as 1.4% O_2. Results obtained with synthetic air made by mixing O_2, N_2, and CO_2 were identical to atmospheric air. Although 5% O_2 delays senescence by about 30 d, there is no increase in mass of either pods or seeds of plants harvested at 112 as opposed to 83 d. No permanent photosynthetic metabolic changes were found; for example, the CO_2 compensation point of leaves of soybean plants grown under 5% O_2 was essentially normal when measured in air. A preliminary experiment with wheat, another C_3 plant, indicated similar inhibition of seed development by sub-atmospheric concentrations of O_2.

Exposures of soybeans to 5% O_2 during only certain specific stages of development indicated two critical periods in which O_2 concentration affected reproductive growth. Plants treated from 14–35 d of age, the period of rapid vegetative growth up to mid-flowering, produced large numbers of shortened pods which contained an average of about one seed per pod which was larger than normal. Plants treated from 35–50 d, the period of early seed development, produced large numbers of pods of normal size, but the pods contained only incompletely developed seeds. Late exposures from 50–83 d, the period of pod filling, did not affect pod development but inhibited seed development. Thus early exposures seem to arrest pod development while mid-term and late exposures arrest only seed development.

In sorghum the number of fully developed seeds is also decreased by all levels of sub-atmospheric concentrations of O_2 whereas the morphology, time of flowering, and dry weights of the aerial portion and roots are not significantly altered (Table 2). Similar results have been obtained in two separate experiments.

Table 2 Effect of Sub-atmospheric Concentrations of O_2 on Martin Milo Sorghum Growth

	% O_2			
	5	10	15	21
Dry wt (g)				
Leaves and stems	57.5	47.6	53.2	66.7
Seed head	5.9	8.5	9.1	16.8
Seeds	0	1.6	1.7	7.7
Roots	38.2	41.1	30.9	43.2

Measured at 168 d of age with aerial portion of the plant exposed to indicated gas phase from 67–168 d. (See Table 1 for experimental procedures.)

By contrast with the beneficial vegetative growth results previously obtained under sub-atmospheric O_2 concentrations in short term studies on C_3 plants[1], our long term studies demonstrate that O_2 concentration is a key factor controlling reproductive growth and the use of O_2 limitation to increase the efficiency of photorespiratory plants is ineffective for seed producing crops. This control role of O_2 requires higher levels of O_2 than mitochondrial respiration. The rate of dark respiration in soybean leaves[3,4] is the same at 1–3% and 21% O_2 while our results demonstrate that the optimal level of O_2 required for maximal reproductive processes approaches, or even exceeds, atmospheric concentration and reproductive growth may even be favoured by super-atmospheric O_2 concentrations. The nature of the O_2 receptor involved in reproductive growth is not yet identified although systems such as ascorbic acid oxidase and flavoenzyme oxidases are known

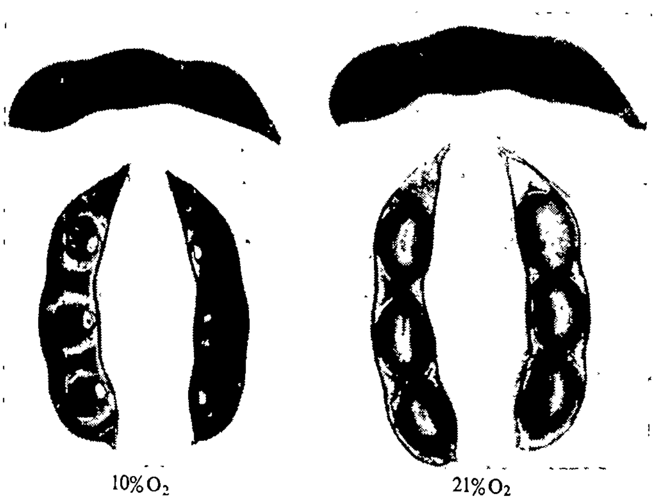

Fig. 2 The effect of sub-atmospheric O_2 on seed and pod development of soybean exposed to 10% O_2 from 14–83 d in comparison to 21% O_2 (air).

to require higher O_2 concentrations than the cytochrome systems of dark respiration[5]. On a physiological basis it seems that an interaction of O_2 with the translocation of photosynthate to the reproductive sinks such as the seed occurs and this may be mediated by an effect of O_2 on the amount or specific activity of endogenous hormones such as auxins, gibberellins, cytokinins, or ethylene. The ability of exogenous hormones to overcome the effect of low O_2 is being tested. It is also noted that the root becomes a more effective sink as the reproductive parts become less effective.

The Earth's atmosphere has altered considerably during its evolution, the O_2 concentration increasing from zero to its present 21%; our data suggest that plants reproduced by seeds may not have evolved until a later stage when the build-up of O_2 in the atmosphere was close to its present proportion.

The requirement for high amounts of O_2 for normal reproductive growth may have ecological significance. At high elevations the amount of O_2 is substantially decreased from that occurring in air at sea level. Seed yields of plants grown on elevated plateaux may be decreased (Allred, R. C., personal communication). On the other hand, vegetative growth and root growth may be improved.

Our observation permits a new experimental approach to elucidate factors controlling plant reproductive development. The occurrence of the O_2 effect in plants with high and low rates of photorespiration suggests that it may be common to a large number of higher plants. Elucidation of its mode of action may provide information useful in increasing seed yield of grain or alternatively vegetative growth of forage or root crops.

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Increased Yield through Correction of Sulphur Deficiency in Ryegrass exposed to Sulphur Dioxide

SULPHUR dioxide (SO_2) in the air at high concentrations may be injurious to higher plants: at low concentrations it is not injurious. On the basis of experimental exposures[1], a concentration of about 429 µg SO_2 m^{-3} (0.15 p.p.m.) has been widely accepted as a threshold below which a range of higher plants would not be injured even during prolonged exposure. The dividing line between injurious and non-injurious concentrations is not well defined because plants vary in their susceptibility to injury according to the species, stage of growth, environmental factors, such as light intensity, humidity, water supply, and the duration of the exposure at a given concentration[2]. The injurious effects of SO_2 have been investigated extensively but much less attention has been given to its effects at non-injurious concentrations. This aspect deserves further study because in some circumstances the effects of SO_2 are known to be beneficial. Thus, it has been observed that (i) the rate of photosynthesis in lucerne, as measured by the uptake of carbon dioxide, was increased by exposing the plants to air containing 486 µg SO_2 m^{-3} for 66 h (ref. 3) and (ii) the yields of dry matter of several species growing in solution culture were enhanced when the shoots were exposed for 9 to 20 days in air containing SO_2, labelled with ^{35}S, at 200 and 500 µg m^{-3}; ^{35}S appeared in both organic and inorganic fractions of the plant sulphur[4]. The fact that SO_2 can be utilized after absorption through the shoots may be of widespread significance in crop nutrition in industrialized countries. Indeed, we have found recently[5] that when ryegrass was grown on sixteen soils from England and Wales in a controlled environment cabinet with air filtered to remove SO_2, the plants developed sulphur deficiency on all soils except one; deficiency has not been encountered in crops growing on these soils in the field. The inputs of sulphur from the air and rainfall to plants and soils under field conditions are not known with certainty, but in rural areas in Britain the air near the ground contains on average 50 µg SO_2 m^{-3} (ref. 6) and for urban areas the average is slightly above 100 µg m^{-3} (ref. 7).

We report here the results of an investigation into the effects of prolonged exposure to SO_2 on ryegrass (*Lolium perenne*) variety S23, when grown on a soil known to be unable to meet fully the plant requirement for sulphur[8].

The experimental soil, which was taken from an area under grass/arable rotation at the Grassland Research Institute, has been described elsewhere[8]. Ryegrass was sown in sixteen polyethylene-lined pots containing 6.26 kg soil (oven dry basis) which received additions of nutrients as follows: 150 p.p.m. nitrogen (as $Ca(NO_3)_2$); 20 p.p.m. phosphorus (as $CaH_4(PO_4)_2$ plus KH_2PO_4); 5 p.p.m. magnesium (as $MgCl_2$). Eight pots of soil received 61 p.p.m. potassium either as KCl, KH_2PO_4 plus K_2SO_4 to give 10 p.p.m. added sulphate-S, while the remaining eight pots (without added sulphate-S) received the same amount of potassium simply as KCl plus KH_2SO_4. Initially, all pots were housed in a 'Saxcil' controlled environment cabinet with air filtered through activated charcoal to remove SO_2 (cabinet 1). After emergence, the seedlings were thinned to seven per pot, and the surface of the soil was covered with a 1 cm layer of opaque polyethylene granules to minimize evaporation. The pots were weighed at 2-day intervals and distilled water was added to bring the soil to 75 cm water tension (about 1/3 bar). Cumulative water losses were computed to provide an estimate of transpiration. The plants were grown at 23° /18° C day/night temperatures with light intensity of 110 W m^{-2} and a 16 h photoperiod. Air, with supplementary CO_2 to maintain a level of 300 p.p.m., was pumped into the cabinet after filtering and was continuously circulated; there were six apparent air changes per hour, the flow rate across the plants being about 20 cm s^{-1}. At 25 days after sowing, one set of pots, comprising four with and four without added sulphate-S, was transferred to another 'Saxcil' cabinet set to give the same environmental conditions as in cabinet 1, except that SO_2 was added to the air at the inlet at a controlled rate (cabinet 2). The concentration in the cabinet was measured each day, usually on two occasions, by a modification[9] of the West and Gaeke method. The SO_2 flow rate was adjusted so that the mean concentration in the cabinet was 131 µg m^{-3}. The air in cabinet 1 contained no detectable SO_2.

The ryegrass was harvested at 17 days after the pots were divided between the two cabinets (harvest 1), and again at 38 days (harvest 2) and 59 days (harvest 3). At the first two harvests the shoots were cut at 2.5 cm above the soil, but at harvest 3 they were cut as close to the surface of the soil as possible and the roots were separated from the soil by sieving and washing. After harvests 1 and 2 nutrients were applied again at the original rates.

In cabinet 1 the plants that were grown without added sulphate-S became deficient in S after the first harvest. They were paler green than those grown with added sulphate-S and

Table 1 Yield and Sulphur Content of Perennial Ryegress Grown in Filtered Air Containing Nil or 131 μg SO_2 m^{-3}

			Shoot dry weight† and S content‡						Root dry weight† and S content‡	
			Harvest 1		Harvest 2		Harvest 3		Harvest 3	
Cabinet No.	SO_2 added to air (μg m^{-3})	Sulphate-S added* (p.p.m.)	Dry weight (g)	S content (%)	Dry weight (g)	S content (%)	Dry weight (g)	S content (%)	Dry weight (g)	S content (%)
1	Nil	Nil	11.7	0.26	11.5	0.12	8.7	0.08	8.5	0.11
		10.0	11.2	0.28	16.2	0.31	19.3	0.28	8.7	0.16
2	131	Nil	10.0	0.34	14.7	0.31	14.0	0.17	8.3	0.13
		10.0	10.4	0.37	15.5	0.40	16.2	0.35	7.5	0.17
Standard error (12 df)±			0.31	0.009	0.67	0.013	1.08	0.015	0.50	0.007

* Addition per pot on each of three occasions, namely before sowing and after harvests 1 and 2. † Dry weight per pot. ‡ Dry matter basis.

were finally distinctly chlorotic. At harvests 2 and 3 the dry weight of the shoots was reduced by 29 and 55% respectively compared with that of plants grown with added sulphate-S; the dry weight of the roots was unaffected (Table 1). In the cabinet with added SO_2 (cabinet 2) there was no evidence of sulphur deficiency, and the dry weights of the shoots and roots of plants grown with and without added sulphate-S were not significantly different.

Results of analysis for total S in the plants using methods described elsewhere[8] are shown in Table 1. The concentration in the shoots of plants that were grown with added sulphate-S did not differ between harvests in either cabinet. But the concentration in the plants that were grown without added sulphate-S declined between harvests. In cabinet 1 the total decline was about 70%, and the concentration at harvests 2 and 3 was below the range of 0.15–0.20% which has been suggested as "critical", that is, just deficient for maximum growth[5,10]. It is notable that the input of SO_2 to the plants in cabinet 2 prevented a decline in S concentration in the shoots between harvests 1 and 2, and that between harvests 2 and 3 it maintained the concentration close to the critical value. In fact, at harvest 3 the S content was, in terms of concentration, twice and, in terms of total amount, three times that of the shoots of corresponding plants in cabinet 1.

The beneficial effects of SO_2, as shown by increases in yield and S content of perennial ryegrass when grown with an inadequate supply of S to the roots, are in marked contrast to a recent report[11] that exposure to SO_2 depressed yield of the same ryegrass variety grown on a soil, the sulphur status of which was not specified. But the fact that the plants were exposed to higher doses (191 μg m^{-3} for 182 days and 343 μg m^{-3} for 63 days) than in our work may provide some explanation for the different effects. The large amounts of nitrogen added and the cutting treatment that we used simulated more closely the conditions under which high-yielding grass is grown as an agricultural crop in Britain. Although the SO_2 concentration that we used may on occasions be found in agricultural areas, it greatly exceeds the 50 μg m^{-3} measured in areas where most of the grass crop is grown. This, and the fact that SO_2 did not depress yields, even when sulphate-S was added to the soil (as in the field through rainfall), suggests that present concentrations near the ground are not injurious to ryegrass in most areas.

Finally, the effect of SO_2 on transpiration requires comment because it has been suggested that, in Britain, many crops exposed to "ambient SO_2 levels" have an increased rate through decreased stomatal resistance[12]. In our investigations, the estimated amounts of water transpired were 10.8 and 11.2 kg per pot in cabinets 1 and 2, respectively. These amounts, which are equivalent to 229 and 234 g water used per g of dry matter produced (see Table 1), do not support the suggestion that SO_2 has a significant effect on transpiration rate, at least in ryegrass.

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Isomerization of γ-BHC to α-BHC in the Environment

Because of the wide use of the persistent insecticide benzene hexachloride (BHC), its residues have been found in soils, plants, animals and foods. From the viewpoint of environmental toxicology, the principal residue problem of BHC is that particular isomers, such as α and β, but not the active γ isomer, accumulate in food[1,2].

Because the insecticidal γ isomer degrades relatively fast for a chlorinated hydrocarbon insecticide, especially in anaerobic conditions such as an aquatic soil environment[3–5], accumulation of α and β isomers has been ascribed to selective metabolism by biological systems, an analogous explanation to that proposed by Ishida and Dahm[6] for houseflies. MacRae *et al.*[7], however, showed that in unsterilized soil the persistence of α, β, γ, and δ isomers was essentially the same.

Newland *et. al.*[8] found both α and δ-BHC in aquatic sediments incubated with pure γ-BHC. These workers have attributed the phenomenon to the differential thermodynamic stabilities among the BHC isomers—chemical transformation of γ isomer to more stable α, δ and β isomer.

To study the metabolic fate of γ-BHC in the environment we incubated highly purified γ-BHC with aquatic sediments under simulated natural conditions and with a soil microorganism under laboratory conditions. Non-radioactive γ-BHC used throughout this study contained 0.0017% of impurity as α-BHC. The radioactive ^{14}C-γ-BHC was purchased from Amersham and Searle Corp., and further purified on a thin-layer chromatographic system until no impurities could be detected by an autoradiographic assay.

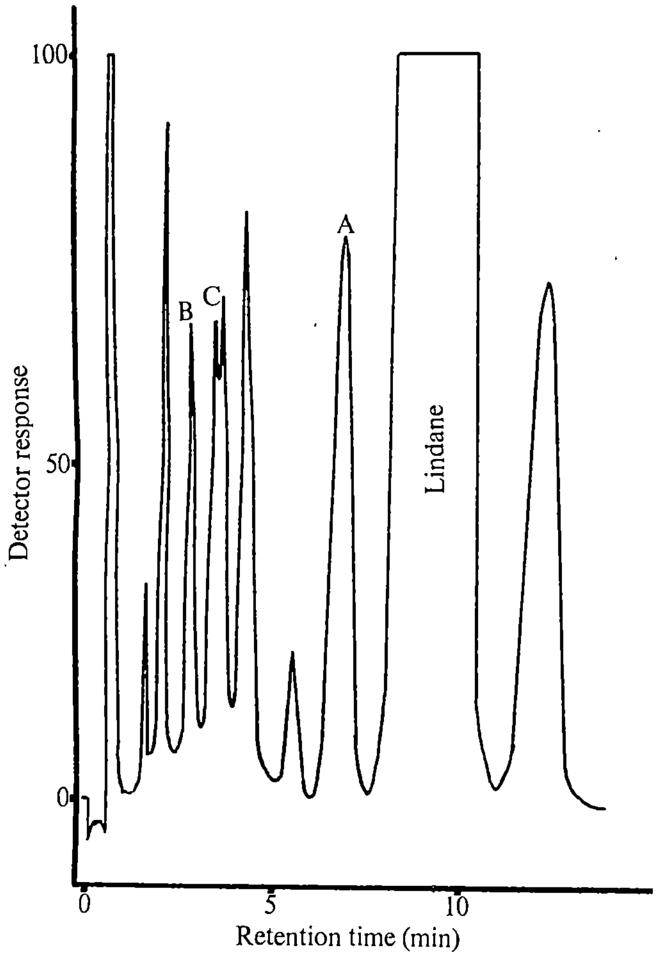

Fig. 1 Sediment from Pearl Harbour, Hawaii, was incubated with γ-BHC for a month. The sediment was extracted, purified, and analysed on a Varian Gas Chromatograph 1800 Series and with an electron capture (^{3}H) detector: QF1, 3.2 mm×1.3 m column at 170° C. A, α-BHC; B, γ-BTC; C, γ-PCCH (lindane=γ-BHC).

The aquatic sediments (*p*H, 7.8; total S, 0.091%; SO_4, 367 p.p.m.; organic matter, 3.4%; sand, 36%; silt, 38%; clay, 26%) were taken from Pearl Harbour, Hawaii, in July 1971, with ambient seawater from a depth of approximately 10 foot. Non-radioactive γ-BHC (100 mg) in 5 ml. of acetone was added to the sediment (approximately 300 g) with 200 ml of seawater. The slurry was transferred into a 600 ml clear, narrow neck medicine bottle which was topped up with seawater. The bottle was sunk to the bottom at the collection site, and was kept there for 30 d.

A portion of the sediment (23.8 g wet weight) was extracted with a 1:1 mixture of chloroform-acetone. A portion of extract was then purified on a 'Florisil' column, 5×65 mm eluting with hexane/ethyl ether (85:15). The results of gas chromatographic analyses (Fig. 1) clearly indicated that the content of α-BHC drastically increased. From the sediment sample (23.8 g) 7.2 μg of α-BHC was isolated (determined by GLC peak areas), a quantity ×4 that found in the original quantity of γ-BHC, even though only a fraction (1/12) of the sediment was extracted. Total recovery of γ-BHC from the sediment was 40%. When untreated Pearl Harbour sediment was extracted and checked on GLC, no peaks were found in the areas with the same retention time as α-BHC (Fig. 1).

For the laboratory study a strain of *Pseudomonas putida* was used. A 48-h-old culture of *P. putida* (2 l) in a mannitol base medium[9] was incubated with 15 mg of γ-BHC and 100 mg of NAD for 4 weeks. Concurrently small scale experiments were conducted using 10 nm of ^{14}C-γ-BHC and 10 ml of a 24-h-old culture of *P. putida* in base medium with and without the addition of NAD (0.2 mg ml^{-1}). The large scale incubation of γ-BHC with *P. putida* yielded approximately 70 μg of α-BHC in a pure form (90% recovery of γ-BHC). This is more than forty times the amount of α-BHC present as impurity in the culture. Examination of the purification product on mass, GLC and thin-layer chromatographic analyses established it as pure α-BHC.

In the small scale experiment *P. putida* was found to produce normally γ-PCCH (γ-pentachlorocyclohex-1-ene) from γ-BHC, but was found to be capable of producing α-BHC on incubation with NAD. The extent of α-BHC formation was 3% in these experimental conditions. Another principal metabolic product accompanying α-BHC was γ-BTC (γ-tetrachlorocyclohex-1-ene), the same end product as Tsukano and Kobayashi[10] recently found in the paddy soils. The extent of γ-BTC formation under identical conditions was 19%. Recovery in these microbial experiments was greater than 90%.

These results demonstrate that α-BHC can be isolated from the environment in quantities larger than those expected as the basis of selective accumulation of impurities from the starting product alone. The cause of the transformation of γ-BHC to α-BHC in the environment could be microbial, for laboratory studies have shown that a microorganism could indeed convert γ-BHC to α-BHC particularly when the medium is enriched with NAD. More direct evidence is, however, needed to confirm this point.

The significance of this work is that we have demonstrated for the first time that microorganisms in pure culture can isomerize γ-BHC. As there is no document to indicate the presence of this type of isomerization mechanism in the chemical or biochemical fields, further studies on the isomerization process are warranted.

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Earthworms and Seeds

EARTHWORMS move seeds[1,2] and viable seeds have been reported in worm casts[3,4]. The causes of loss of seeds from the soil are seldom identified[5,6].

In our first experiment fourteen plant species were selected to provide a range of sizes and shapes of seed (Table 1). Petri dishes were prepared with filter paper to which was added 6 ml. of deionized water and the seeds. One specimen of *Lumbricus terrestris* (minimum fresh weight 3 g) was introduced into each dish. The dishes were kept in darkness at laboratory temperatures for 18 h, after which the worms were transferred to fresh dishes, and the seeds remaining in the original dishes counted. After a further 72 h the seeds in casts were recovered and counted.

Table 1 Fate of the Seeds of the Fourteen Species

	Percentage of offered seed ingested by *L. terrestris*	Percentage of ingested seeds recovered from cast	Percentage of ingested seeds not recovered
Poa annua L.	60	28.3	71.7
Agrostis tenuis Sibth.	50	0.0	100.0
Poa trivialis L.	40	41.0	59.0
Digitalis purpurea L.	32	53.1	46.9
Bellis perennis L.	17	58.8	41.2
Trifolium repens L.	16	62.5	37.5
T. pratense L.	15.5	48.3	51.7
T. incarnatum L.	13	65.0	35.0
Dactylis glomerata L.	10	80.0	20.0
Trifolium striatum L.	7.5	66.7	33.3
Sinapis alba L.	6	66.7	33.3
Festuca pratensis Huds.	4	50.0	50.0
Lolium perenne L.	3	66.7	33.3
Spring barley *cv.* Deba abed.	0	—	—

Results are for twenty replicates, each of ten seeds, for each species.

L. terrestris ingested seeds, but there was wide variation between species, from 0% for barley to 60% for *Poa annua* (Table 1). None of the ingested grains of *Agrostis tenuis* and less than 50% of those of *Poa annua* and *P. trivialis* were recovered from the casts. Survival of seeds of other species was higher.

In a second experiment we studied the germination of seeds recovered from casts. The results show that some seeds can pass through the gut without losing their viability and that the final percentage germination was higher for such seeds than for seeds from the original stock (Table 2).

Table 2 Percentage Germination (12 Days after Sowing) of Three Seeds after Passage of Seeds through the Gut of *L. terrestris*

	Percentage germination		
Species	Original stock	After passage through gut	L.S.D. ($P=0.05$)
Poa trivialis	66.2	90.5	8.60
Bellis perennis	83.0	96.7	8.02
Trifolium repens (non-hard seed)	74.7	82.0	8.67
T. repens (hard seed)	8.3	15.4	4.96

Results of three trials each with twenty replicates of ten seeds.

Viable seeds of many species have been recovered from worm casts by both flotation and direct germination tests.

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Intelligence and Handedness

THE data of Levy[1] have been quoted[2,3] as evidence that people with left handed preferences have significantly lower visuo-spatial IQ than right handed people, although the verbal IQ's of the two groups does not differ. I have analysed data[4] previously obtained and classified for other purposes, to see if they support Levy's conclusions (Table 1).

Table 1 Intelligence and Handedness: Comparison of Data from Two Sources[1,4]

	Graduate science students[1]		Academic scientists[4]	
Numbers (handedness)	10 (L)	15 (R)	13 (L)	132 (R)
Verbal IQ	142 (L)	138 (R)	127.4 (L) (S.E.*=2.65)	128.2 (R) (S.E.=0.57)
Visuo-spatial IQ	117 (L)	130 (R)	120.4 (L) (S.E.=2.61)	120.7 (R) (S.E.=0.78)

* Standard error.
Both studies used the Wechsler Adult Intelligence Scale[5].

My sample of left handed people is small but provides no evidence that there is any difference in IQ components between left and right handed people.

Neither of these investigations is on an adequate scale and both are on specialized samples. It therefore seems premature to reach any conclusions about relationships between IQ or IQ components and handedness.

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BOOK REVIEWS

Space Science

Space Physics and Space Astronomy. By Michael D. Papagiannis. Pp. xiv+293. (Gordon and Breach Science: New York, Paris and London, 1972.) £7.70.

LATE in 1957 the Russians astonished the world by creating the first artificial satellite of our Earth and by following it almost immediately by the incarceration of a dog in an orbiting death cell large enough for the world to view. Everyone will remember the successive disappointments suffered at that time by the Vanguard project and, compared with the weight of the first three Sputniks, the pathetic little Explorer I launched at last on January 31, 1958. Yet this tiny satellite with its Geiger counter payload rather than the living passenger of Sputnik II provided both a pointer to the main road to be taken by space science and the most important single discovery of the space age. By its unexpected detection of the trapped radiation, to which Singer and Christophilos had been giving theoretical consideration, this payload started the satellite study of the Earth's environment and its relationship to the Sun which has been the first great contribution of spacecraft techniques to our understanding of our world. Cosmic X-ray astronomy grew out of solar X-ray studies which were themselves part of the field of solar–terrestrial relations. To this branch of astronomy I would assign the next place in the great achievements of space science. None of this is to underrate in any way the important science coming out of manned and unmanned lunar expeditions and the instrumented flights to Venus and Mars. These, however, have not had the tremendous integrating effect which solar–terrestrial studies have had on our picture of the Earth's atmosphere, ionosphere, magnetosphere and polar phenomena in relation to the Sun, its activity, radiation, wind and high energy particle emissions. Nor do they appear to have the potential significance of our introduction to new, energetic and sometimes transient celestial phenomena through the X-ray window on the Universe. The unexpected array of cosmologically significant phenomena from black holes to intergalactic matter and from supernova remnants to interacting binaries is comparable only to the prospects opened a decade or so earlier by the sister branch of radio astronomy.

Merely to mention radio astronomy, X-ray astronomy and so on is to remind oneself that space astronomy, space physics and indeed the whole of space science are not disciplines of science but branches of scientific disciplines marked out by the highly sophisticated technology involved. *Space Physics and Space Astronomy* sets out to introduce the graduate considering a career in space science or the professional scientist interested in it to the field as a whole. In doing so it, perhaps of necessity, largely ignores the technological basis and often even the experimental basis of the subject and reviews with readability and skill the basic geophysics, solar physics, planetary physics and astrophysics which have been the hunting ground of space research. In spite of some charming infelicitations of expression betraying perhaps the Greek background of the author and some less excusable faults in proof reading the book remains one of the best introductions to the subject I have seen. Papagiannis is, himself, a notable worker in many of the areas he describes so well. Personally I could wish for a little more discussion of the experimental procedures which entitle the subject to the adjective *space* and it is noticeable that the mathematical analyses cluster round the author's own areas. Some errors of fact are obviously mistakes of expression, but I wonder if anyone still believes Cygnus–A to be a colliding system. *Space Research* is a book intended for engineers. It would have been far better to use the translator's effort in providing Papagiannis' book for Russian engineers. Part 1 is so full of errors as to be useless, even if the engineers did need to know so much geophysics and astrophysics to design spacecraft. Part 2 is concerned with the effects of space environment on materials. Like Part 1, on the whole it lists empirical data without engendering understanding. No doubt this matters less in designer's reference material but there is the somewhat unfortunate disclaimer on page 3 "The data presented here should not be used as a standard, and they will as a rule only give rough evaluations".

R. L. F. BOYD

Biochemistry of Ataxia

The Pharmacology of Extrapyramidal Movement Disorders. By H. L. Klawans, Jr. Pp. vi+137. (S. Karger: Basel, London and New York, 1973.) 49 Sw. francs; £5.75; $13.75.

BASIC biochemical and pharmacological discoveries led to the prediction that levodopa would help patients with Parkinson's disease, a prediction that has been amply fulfilled in clinical practice. A side-effect of levodopa treatment is the production of abnormal movements reminiscent of those seen in diseases characterized by chorea and torsion dystonia. Naturally clinicians and basic scientists have seized on this observation as a clue to the pathogenesis of such disorders and in recent years many studies on the clinical and animal pharmacology of extrapyramidal movement disorders have been published. Dr Klawans's monograph is timely, for it draws together much of the presently available information on this topic.

The central theme of the book is the disorder of dopaminergic and cholinergic mechanisms that underlies Parkinson's disease. This is described at length from the viewpoint of a clinician who starts with observations in the clinic, and then attempts to explain them on the basis of what is known about dopamine and acetylcholine in the brain. The reasons why levodopa works (or doesn't in some patients) and the causes of the side-effects it produces are discussed at length. The value and mechanism of action of other antiparkinsonian agents are also reviewed, as is the value of levodopa in other akinetic-rigid syndromes, such as olivo-ponto-cerebellar degeneration, progressive supranuclear palsy, manganese poisoning and Wilson's disease.

Dr Klawans then turns to abnormal movement disorders such as the tardive dyskinesias produced by neuroleptics, Huntington's chorea and other choreiform states, and torsion dystonia. Building on the framework of the pharmacology of parkinsonism, he suggests that some of these abnormal movement disorders are due to over-stimulation of some dopaminergic receptors in the corpus striatum. Inevitably, much of the section is hypothetical, but the clinical, pharmacological and other evidence is marshalled in a convincing way to support the author's tentative conclusions. The hypotheses that Dr Klawans, and many other workers in the field, have put forward to explain abnormal movement disorders on a biochemical basis provide a framework for further research. Time will tell whether they are true or not, but meanwhile Dr Klawans's monograph provides a useful statement of the present evidence and will be of value to the many clinical and basic research workers interested in this expanding field of neurology.

C. D. Marsden

Solid State Theory

Theoretical Solid State Physics. By Albert Haug. Vol. 1. Pp. xiv+497. (Pergamon: Oxford and New York, May 1972.) £7.

Theoretical Solid State Physics. By Albert Haug. Translated by H. S. H. Massey. Edited by D. ter Haar. Vol. 2. Pp. xiii+355. (Pergamon: Oxford and New York, August 1972.) £6.

These volumes are translations from the German of the original editions, of which volume 1 was published in Vienna in 1964 and volume 2 in 1970. The level of treatment is suitable for a student who has completed a first degree in physics. It was the aim of the author "to make the derivations so detailed that they could be understood without any additional calculations". This aim, it must be said, is on the whole achieved. Formal mathematical derivations are set out at length, to the extent that the summation of a geometric series may be broken down into several steps. One can only presume that it was for this reason the book was thought to be sufficiently different from existing texts to merit translation. The market is, after all, well supplied with introductions to solid state theory.

The intended scope of the volumes is perhaps indicated by a quotation from the foreword to volume 2: "We are thus dealing with a closed body of solid state physics which will not be rendered superfluous by yet newer developments, and this state is represented by the present work". Volume 1 covers the elementary theory of electrons in a perfect lattice, including Wannier functions but with only a sketch of methods of band-structure calculation, the Bohm and Pines theory of the interacting electron gas, simple treatments of the magnetic and dielectric properties of solids, and the elementary theory of lattice vibrations. There is some emphasis on one-dimensional models. Volume 2 treats the electron-lattice interaction, with an account of Bardeen's classic 1937 calculation, gives a rather formal account of transport phenomena based on the Boltzmann equation, and concludes with sections on semiconductor theory and luminescence.

Even in 1964, however, volume 1 might have been thought a little out of touch. (The only updating I can find is a footnote on page 177.) Volume 2 virtually ignores the insights and results that have been gained in the past decade. A student studying these volumes in isolation would gain the impression that it is almost impossible to make calculations in solid state physics with more than order of magnitude accuracy. It was, to take one example, already clear in 1964 that the concept of a screened model potential was a valuable aid in understanding the properties of simple metals, and making realistic calculations. The Bohm and Pines theory, and the Bardeen theory of the electron-ion interaction, are landmarks in the development of solid state theory, but the equivalent dielectric constant formalism is at least as easy to present, and has proved far more convenient in practice.

It is impossible to recommend these volumes as an introduction to the current literature of the solid state. They have, perhaps, some value as a reference work for students who have local difficulties in the mathematical derivations of elementary solid state theory.

D. A. Greenwood

Geochemistry

Geochemical Exploration 1972. Edited by M. J. Jones. (Proceedings of the Fourth International Geochemical Exploration Symposium held in London April 17–20, 1972. Organized by the Institution of Mining and Metallurgy. Pp. xiv+458. (The Institution of Mining and Metallurgy: London, February 1973.) £12; $30.

This volume contains thirty-seven papers read at the Fourth International Geochemical Exploration Symposium held in London in April 1972. They are the work of forty-four authors from twelve nations and encompass a great number of the more important aspects of the subject, though, whilst including some short case histories, they are mainly concerned with the results of research into new and developing fields. The publishers are to be congratulated on the speed with which they have produced this volume. Apart from a few over-reduced and unreadable figures, the reproduction and legibility of figures both black-and-white and coloured are of a high order.

The volume demonstrates the rapid advances being made in applied geochemistry and includes a number of "firsts". One is a paper describing anomalous metal concentrations in snow overlying ground containing sulphide ore. Another is a novel account of geological mapping and geochemical sampling using light aircraft. The methods of scooping up rock and sediment samples during flight at only a metre or so above ground level are enough to make the reader's hair stand on end. Though not quite a "first", the paper by Dr Allan and others on the use of lake sediments in reconnaissance exploration breaks fresh ground in sampling techniques. Other papers concerned with sampling in the more usual media of soil, stream sediment and rock samples cover such diverse topics as the use of gold as a pathfinder element for porphyry copper deposits, trace element patterns as a guide to ore-bearing granites, sulphur and nickel concentrations in serpentinites as a guide to mineralized areas and the use of bedrock geochemistry in regional surveys.

A landmark in the study of secondary dispersion is Professor Govett's paper in which he describes laboratory tank experiments to support the view that significant differential secondary rearrangement of metals in soils and rocks related to sulphide ore bodies may occur under the influence of electrical potentials. This could lead to new approaches to exploration through various electrophysical and chemical measurements which would allow a deeper penetration of thick transported overburden—a topic dealt with in other papers. Statistical analysis of geochemical data is given prominence in six papers and is applied in many others. Cluster analysis, pattern recognition and automated colour-mapping are included.

Mercury as a pathfinder element has attracted much interest in recent years, and it is interesting to read that studies of mercury in rock samples from an Irish deposit indicate that mercuric sulphide may be a better guide to ore than total mercury values. The use of gases is described in two contributions from the USSR which *inter alia* demonstrate their value in mapping fracture zones.

About one third of the papers deal with new techniques and instrumentation. These include the measurement of atmospheric mercury with a Zeeman spectrometer, a microwave-induced argon plasma emission system for trace analysis, fission track radiography, neutron activation analysis, *in situ* measurement of Eh and *p*H in diamond drill holes and the use of the ion-sensitive fluoride electrode.

A. M. Evans

NATURE Volume 243
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Nuclear Fusion or the Sun God?

ONCE upon a time it was predicted that the population of the world would breed itself to death within a few years. Fortunately, or it might even be said predictably, this has come to nothing. Now, yet another series of predictions are being generated which will have equally apocalyptic consequences if they come to fruition. To put them in their simplest form it is that there is a shortage of energy and that there is no foreseeable way in which the world can meet its energy demands once the twenty-first century begins.

The wheel has certainly turned full circle. It is now presumably predicted that a gradual cold death will face the population of the world once the energy resources are exhausted. Only a short few years ago the concern being expressed was that the excess heat generated by more and more power stations, presumably straining to their utmost to meet the ever increasing demands of an ever sophisticated society, combined perhaps with aircraft flying in the stratosphere which could alter the radiation balance in the atmosphere, would ensure that the temperature would rise to such an extent that life would gradually become unbearable. It is no wonder that mere onlookers of the environmental scene are bemused.

But the true picture, of course, lies in between the two extremes as painted here. There is indeed an energy problem but it is far from being a crisis. It is now up to responsible governments to insure for the future by devoting reserves towards long-term research on energy problems. A series of three programmes entitled *The Energy Crunch* on BBC Television which came to an end this week has done a little to put the British viewer in the right frame of mind for the future, although it is to be hoped that viewers saw all three programmes the last of which went some way to redressing the unfair balance of the first two. The BBC must be commended for many of its science programmes for it has on occasion conspicuously failed to provide unbiased and logical argument on many issues. The recent series of programmes can also be criticized to some extent in this respect.

The repeated assertions that the safety of nuclear power plants is in doubt, without repetition of the impressive safety record of the British nuclear power industry, was one of the most insidious aspects of the programme. It must be considered to what extent the seemingly deliberate destruction of nuclear power as a solution to the energy problem was motivated by the *raison d'être* of the programme makers. How indeed could such a strong representation be made for solar power and geothermal power if fission reactors, both the present generation and the fast breeders, had been shown to be what they are, the workhorses of the nuclear power industry in the coming years?

It must also be asked why it was that much more emphasis was placed on solar energy rather than energy from fusion as the basis of man's hope for the future. Fusion research is at an advanced stage and although it cannot be said that such reactors will prove to be the salvation of mankind—many years of research are needed before such categorical statements can even be contemplated—more money can safely be placed on the probability of fusion providing a safe, economical and regular supply of power than on any system designed to derive power from the sun.

But the makers of *The Energy Crunch* are correct in one respect and that is that speculative ideas for generating energy on a large scale deserve attention. Solar energy is only one of these possibilities the potential of which was reported by a combined NSF and NASA panel at the end of 1972. The panel reported that the total energy requirements of the United States in 1969 for example could have been supplied by the sunlight incident on 0.4 per cent of the country's land area. But what of coal? Is there not a possibility that power can be extracted from this source by combustion underground thus saving the cost of mining? Cannot nuclear power be used to electrolyse water in order to produce a supply of hydrogen which is a readily transportable source of energy? It is only to be hoped that the energy laboratories, energy departments and energy units which have mushroomed all over the world in what seems like recent months will not only devote their time and efforts to estimating, predicting or projecting the future of our existing energy resources but that the right people within these institutions will be given their heads, and the finance, to pursue futuristic ideas.

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100 Years Ago

Winters and Summers

A FRIEND writes to me :—"From my observations of climate here (Belfast) I should say that I never saw a severe winter followed by a really fine summer. The severest winters I remember were those of 1854-5, and 1859-60. The summer of 1855 was very wet, and that of 1860 deplorable. The finest summers I remember were those of 1842, 1857, and 1868; in every case the preceding winter was very mild."

I would add to this, that the severe winters of 1865 and 1870 were not followed by remarkably fine summers. The harvest weather of 1866 was unusually bad.

Can any of your readers throw light on this subject from carefully kept registers? JOSEPH JOHN MURPHY

Old Forge, Dunmurry, June 6

from *Nature*, 8, 182, July 3, 1873.

OLD WORLD

Whaling Commission still in Trouble

THE International Whaling Commission celebrates its quarter centenary this year. And trailing its past history of decisions taken too late or not taken at all, the commission is in London again to debate the future of the whale.

The scene is much like last year's. Friends of the Earth are picketing, the press is clamouring to be let in, the scientists are cautiously producing their advice, and the commissioners are there to ensure that whaling continues.

The proposal for the ten year moratorium on all whaling will be discussed again and rejected again, the commission will agree to make further efforts to get the whaling countries that are not members of the IWC (Brazil, Portugal, Spain, Chile and North Korea) to join, and more noises will be made about strengthening the commission's machinery (a permanent secretariat will almost certainly be established). At the end of the day some progress will have been made.

As the week began the scientific committee had still not completed its assessment of the current situation, nor produced its recommendations. But it is plain that the committee will come out against a total moratorium (so long as certain species are protected there is no harm in exploiting healthy stocks, it argues), but it will suggest that the fin whale be better protected.

The fin is the largest of the baleen whales that the commission members still hunt. Quotas set for it in the Antarctic and North Pacific for the 1972–73 season were 1,950 and 650 respectively, a catch that was exceeded in the North Pacific by 108, but not met in the Antarctic by 189. Stocks of the whale are heavily depleted, and it is calculated by the scientists that the species will have to be left unfished for 23 years for it to return to its maximum sustainable yield (MSY)—the maximum number that can be taken from any population without depleting the stocks.

At present the scientists estimate that 3,000 fins could be taken in the Antarctic without damaging the stocks further, but many more could be harvested if the fin is allowed to recover unhunted. It is therefore on the cards—although rather an outside possibility—that the conference will agree to a moratorium on the fin whale.

Dr Robert White, Director of the United States National Oceanic and Atmospheric Administration, opened the bid for a moratorium on all whaling on the first day of the meeting. He went on to say that "placing the fin whale under a complete moratorium is the most urgent matter before this commission". Mr Michael Stodart, Minister of State for Agriculture, Fisheries and Foods, also put the weight of the British delegation behind the moratorium on the fin whale. Without actually calling for a ban in so many words, he said "if this species is to recover to its maximum sustainable yield as fast as we would wish, the commission must be prepared to take much more stringent conservation measures. Indeed we believe that this is the species with the strongest case for a moratorium".

The scientific committee's views will not actually include a recommendation

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POLLEN

Keep on Counting

IT'S been a good year for pollen and a bad one for hay fever so far. But, in spite of the estimated three million sneezing individuals spread across Britain, the pollen count has not been as high this year as it has in the past.

This year's record count to date—on June 19—rose to 351, the highest level for two years. But the largest figure in the records of the Asthma Research Council, which faithfully keeps the daily count from the roofs of Paddington, is a massive 820, recorded on June 17, 1964.

The pollen that causes all the trouble is grass pollen. Records are also kept of the amounts of tree, flower and nettle pollen in the atmosphere, but they are not published daily. Relatively few people are allergic to them. But some three million people are allergic to grass pollens—and nobody knows why. There are, literally, thousands of different allergies found, but grass pollen is the commonest. Of the unlucky three million, some 0.5 million need medical treatment. Once the particular pollen or pollens that are setting up the allergy have been identified (no easy task as there are hundreds of different grasses), a desensitizing vaccine can be given and the suffering eased. Which is of little comfort to the majority of those allergic, who snuffle away all summer whenever the pollen count creeps up around the 100 mark but who aren't ill enough to go to a doctor.

But although this year does not hold the record for a high pollen count, the levels found during the recent heatwave were unusually high, particularly for mid-June. Normally pollen is released from early June to August, and the count tends to rise steadily, peaking in July. This year, however, damp weather in May and early June held the release of pollen back, and when the heatwave arrived the pollen sacs in the grasses were loaded, so that when the Sun finally burst them more pollen than usual was released at one time. One reason why so many people who normally do not suffer from hay fever were affected this year may be the speed at which the pollen count rose, defeating the body's immunity response which can build up resistance to the pollen if the quantities in the atmosphere accumulate slowly.

Nonetheless, the pollen count is highly elastic. Once the pollen is in the atmosphere it tends to stay there until it is flushed out by heavy rain. Thus the count was 351 on Tuesday 19, but only 3 on Thursday 21 following the heavy rain of last week. Friday's warm Sun had the count up to 69 again. Light showers will do little to shift the pollen in the air and will only encourage the grasses to produce more.

The count is taken with a spore machine that takes air in against a sticky microscope slide that revolves once every twenty-four hours. The pollen sticks to the slide and the grains are counted at noon each day, with the final count being the average number of grains found per cubic metre of air over twenty-four hours.

Although the Asthma Research Council's count is actually taken in London, it is held to be reasonably accurate for the whole of the home counties as pollen is so light that it can be blown miles once it has been released.

The Asthma Research Council counts the pollen largely because hay fever is a closely related disease to asthma. The council spends £41,000 a year on asthma research, including in its work studies on hay fever and other allergies. Research has revealed that the excreta from house dust mites are a chief cause of asthma; the researchers have also come up with the rather depressing conclusion that while many adolescents grow out of hay fever, a number of adults progress in middle age from hay fever to asthma.

for a moratorium. All the committee will do is outline what will happen if various courses of action are taken.

The best that can be hoped for is that the quota on fin whales will be further reduced, that firm quotas will be established for other species—minkies and sperm whales in particular, that a permanent secretariat, including a number of scientists, will be established, and that a more successful attempt will be made to bring the non-IWC members into the camp.

Some improvement in monitoring the number of whales caught during the season would also be welcome. Monitoring is at present the task of the International Bureau of Whaling Statistics. This year when it set the date for the end of the season, the rate of catch promptly doubled, so that 745 minkie whales over the 5,000 quota set in the Antarctic were taken.

It is in any case as well that a total moratorium on whaling will not be declared by the commission. The IWC has no hold over its members other than the hold of public opinion. Although Japan has been party to agreements to limit the numbers of certain species taken, she has still, for the past four or five years, taken whales outside her quota using a whaler sailing under a Brazilian flag (Brazil not being party to the IWC). The commission's constitution even allows for commission members to flout the commission's agreements, while still remaining members. If a total moratorium were to be imposed, it is more than likely that Japan and the USSR would ignore it, thereby destroying the IWC.

OECD

Research Efficiency

THE scientific standing of the smaller countries in Europe "is out of all proportion to their comparatively limited resources" according to the Organization for Economic Cooperation and Development. But several arguments suggest that "this very high grade research effort (cannot) be profitably continued, particularly in university laboratories, purely by following the main trends of international scientific activity as in the past".

This verdict emerges from the second of the OECD's expected three volume analysis of the research systems in the major European countries and North America. The countries involved in the present study are Belgium, Netherlands, Norway, Sweden and Switzerland. (*The Research System*, Volume 2, OECD, £2.38.)

The current volume is the successor to the report in which the research council system in Britain was compared with the funding organizations for science in France and West Germany. The patterns of research in the two sets of countries are very different, and this is based, the report says, in the conduct of scientific and technological activities particularly in relation to economic activities. As the report points out, in the set of countries analysed in the present report "whatever may have been the constraints of international competition, research and development policies have pursued a steady path." This contrasts sharply with the progress of science in Britain, France and West Germany which has developed according to a "saw tooth pattern full of sudden changes and sharp breaks".

The report states that the "scientific area which the smaller countries occupy in the mass of knowledge produced throughout the world seems far greater than their 'economic space'." But the OECD examiners feel that the high grade research effort present in these countries cannot continue profitably, particularly in university laboratories. They quantify their argument by pointing out that:

- As the volume of world research continues to increase so will the number of branches of research. If these countries attempt to follow every line then "scientists in countries of limited resources will court failure by dispersing their efforts".
- Several fields of research, especially in physics, are particularly attractive to researchers but their economic value is dubious and the research tends to be expensive.
- Considerable capital expenditure is needed in many scientific sectors where research cannot make further progress without much capital and without setting up large teams.
- International technological competition will force nations to make choices and to specialize more narrowly in the industrial rather than in the pure research sector.

The OECD, in its own way, advises these countries that little progress can be made in the future without scientific effort being based on an overall strategy or, in other words, a national policy for research. Britain, of course, does not subscribe to this view and the OECD report does acknowledge that the need for countries to work out national research strategies is not always economically urgent.

Readjustment is the key word for these five countries, according to the report. None of the countries has assumed responsibility for large scale technological development — like the large industrialized nations—and so the difficulties of readjustment are not as large as they could be. Resources can be reallocated without large scale redundancies among scientists and engineers.

But the real challenge is yet to come. The capacity of these countries for readjustment in the industrial fields has already been shown, but will they succeed in harnessing this capacity with the same energy in the solution of their major socio-economic problems? The crucial question, according to the report, is whether "they will be able to equip themselves with institutions devoted to scientific research which are capable of making the most rigorous and essential choices and yet avoid paralysing unofficial initiative with red tape." The OECD, this time, has hit the question right on the head.

ROYAL SOCIETY

Financial Problems

THE Royal Society is to double its fellowship fees. At a recent meeting of the fellowship it was agreed that the annual subscription fee will go up from £10 to £20. This decision has still to be ratified by July 12's council meeting and the society's statutes will have to be changed before it comes into effect.

The reason for the rise is simply impecunity. In spite of the large grants that the Royal Society handles from Parliament, the society's general purposes fund, which is used to run the society's own business, has fallen into deficit. The account was £100,000 down last year, double the deficit in 1971.

The situation is being aggravated by the fact that many of the covenants that the Royal Society received in 1966 and 1967 just before it moved to Carlton House Terrace are now running out. These covenants produce an income of £50,000 a year. The society has also been hit by the £30,000 loss made on its publications last year, the first loss recorded for 18 years.

Sir David Martin, the Royal Society's secretary, said this week that doubling the subscription "will only be a small help". The society only has just over 850 fellows, so that an increase of £10 a head will only raise something under £9,000. But, says Sir David, "by beginning with an increase in the fellows' subscriptions, we are at least showing that we are willing to help ourselves".

Like many other scientific bodies — the Royal Institution for example, which has largely weathered its immediate financial problems (see *Nature*, **243**, 3; 1973)—the Royal Society has been hit by inflation. The society has always tried to keep its publications work at just better than break-even, with price increases infrequent. The cost of some of the society's publications has already gone up this year—the *Proceedings* for example—and the society is clearly going to have to make a little more profit in future from publications in order to help make ends meet. A further appeal to help redress the general purpose fund's deficit is also likely.

Scientific Agreements Signed in the United States

from our Soviet Correspondent

THE agreement signed last week between the United States and the Soviet Union on cooperation in agriculture, transportation, ocean studies and cultural and scientific exchange, together with the agreement on nuclear arms limitation and the peaceful uses of atomic energy would seem, at first sight, to be a major step forward in scientific and technical cooperation between the two powers.

But a great deal of the ground covered is not so much an innovation as a ratification in greater detail of the Soviet–United States agreement of May 24, 1972, which left room for further cooperation between the countries.

Cooperation in oceanography has already begun with such projects as the two month long meteorological survey of the Bering Sea (*Nature*, **241**, 420; 1973), or is well into the planning stage as in the case of the joint Glomar Challenger mission (*Nature*, **242**, 289; 1973). A preliminary cooperation programme in marine pollution has already been drafted and the working group dealing with this problem first met in Washington at the end of May.

The new United States–USSR Environmental Commission, under the co-chairmanship of Academician Fedorov of the USSR and Dr Russell Train, Chairman of the United States Council of Environmental Quality, has already met some ten times this year.

Dr Lee Talbot, a senior adviser to Dr Train, said in London this week that the joint commission "has been a success" and added that the new cooperative programme is welcome.

Soviet reaction to the recent scientific agreements takes a double course. For external consumption, the *Novosti* press releases stress the benefit the United States may be expected to gain from the new arrangement. But *Pravda* stresses the advantages to be gained by the Soviet Union.

Thus whereas *Novosti* stresses that Soviet experience in collectivisation might "well prove to be useful" to the United States agricultural industry, an article on town planning (*Pravda*, June 16, 1973) in anticipation of the expected agreements, indicated that cooperation with the United States over "'city environment" would play a considerable part in future Soviet planning.

Regarding future developments the agreement on scientific exchange is at first sight ludicrously small, an annual interchange of "40 graduate students, young researchers and instructors for study and postgraduate research in the natural sciences, technical sciences, humanities and social sciences . . . at least 30 language teachers to participate in summer courses of ten weeks . . . at least 10 professors and instructors of universities and other institutions of higher learning to conduct scholarly research for periods of between three and six months" is all that is specified. But these are minimum figures, and presumably do not include participants in specific programmes otherwise covered. A separate clause also provides for short-term exchanges of academics.

But it may be noted that the British–Soviet exchange arrangements of January 1971 failed to materialize on the scale originally envisaged (see *Nature*, **229**, 482; 1972) so that those visits for prolonged research which did occur became matters for special comment rather than the routine of a working agreement.

The implementation of one clause in the ocean studies agreement could result in acceptance by the Soviet Union of the 10-year moratorium on whaling that is currently under discussion at the International Whaling Conference in London. It is too early to forecast the outcome of the conference, but the large Soviet capital investment in pelagic whaling equipment makes acceptance of the moratorium unlikely. It remains to be seen whether this will be the first clause of the agreement to be broken.

The Nixon–Brezhnev talks have ended in an atmosphere of goodwill, with many references to cooperation already achieved with the progress already made in planning the Soyuz–Apollo project being inevitably cited. In the fields where the interests of both parties coincide, such as the project on oncological and vasculo-cardiac research for example, or where they are not in conflict (general environmental studies) good progress is already being made. The new agreements serve largely to approve and extend these promising beginnings. In cases where interests conflict, however, as in the current whaling issue, the outcome seems far less clear.

LARGE SPACE TELESCOPE

Experimental Definition

by our Cosmology Correspondent

NASA plans to launch a large space telescope (LST) by the shuttle sometime in the 1980s. The telescope will have a 3 m mirror, and may be up to 16 m long and 4 m wide, with a total weight of some 9,000 to 11,000 kg; this makes it one of the most important scientific experiments planned for the 1980s and representatives of four countries have now been chosen to define the experiments which will be carried out.

The LST will be able to examine galaxies 100 times fainter than those seen by the most powerful Earth based optical telescopes, but it is not intended solely to study the remote depths of space. Present immediate plans are for twenty-eight members of the experiment definition team to study proposals covering five areas of astronomy:

- high resolution spectrography
- low resolution spectrography
- imaging optics
- infrared devices
- astrometry

Data handling and operations problems for all experiments are being coordinated by a separate team. There are also five "at large" members of a scientific working group to oversee the development of the project: Lyman Spitzer, Princeton; Arthur Code, Wisconsin; Margaret Burbidge, RGO; John Bahcall, Institute for Advanced Study; and B. Field, Smithsonian Astrophysical Observatory. There can be no doubt that the glamorous astronomical subjects — the energy processes in galactic centres, supernova remnants, QSOs for example—will receive considerable attention. Conventional astronomy will not be ignored, however, and the LST will, for example, provide a good platform for long-term monitoring of atmospheric phenomena on Venus, Mars, Jupiter and Saturn.

A few details of the telescope and its systems have already been decided, as well as the basic size. The guidance system will be accurate to 0.005 arc s ("equivalent", according to NASA, "to locking onto a single strand of hair at a distance of two miles"), and the orbit will be at an inclination of 28.5°, reaching an altitude of 648 to 778 kilometres.

SOVIET JEWS

A Second Letter to an Imaginary Soviet Scientist

DEAR Colleague—Your kind invitation to attend the International Conference in Moscow this summer arrived this morning, and I hasten to reply. The programme is unusually interesting. Yet I am not quite sure whether I shall, in fact, accept the invitation. Something troubles me, and I need your advice as an old friend and colleague.

Let me say, first of all, that I have long wished to visit the Soviet Union, not only for its tourist attractions but in order to make closer contact with Soviet scientists in our field. In an earlier letter (*Nature*, **217**, 123 ; 1968) I remarked on some of the confusions that arise when arrangements are made for Soviet scientists to attend conferences in other countries. If you cannot easily come to us, then we should make special efforts to attend conferences and visit laboratories in the Soviet Union so as to keep each other well informed on our scientific progress.

In particular, I was looking forward to a long visit to your theoretical group, whose head has done such brilliant and exciting work over the last ten years. Imagine my distress, then, to see his name in the newspaper, and to receive from him, by a roundabout route, a heart-rending plea for help and support in terrible circumstances. You know much more about it than I do, for it must have hit you particularly hard that your young colleague, with whom you have worked so closely over so many years, should be dismissed from his post, and from all scientific work, for daring merely to ask that he might be allowed to emigrate to Israel. You and I as scientists, can feel the additional cruelty of the order that his books—the fruits of half a lifetime of effort—should be withdrawn from the libraries and that his papers should not be cited in future.

I understand perfectly that there is very little that you and your colleagues can do in the face of such forces. No doubt you are helping our friend in private, with the gifts and sympathy so characteristic of Soviet intellectuals. I suspect that you will also devise ways by which he can continue to do research in private and will be sure to keep his name in the scientific literature: no scientist who is remotely capable of judging the relevance of a citation would be an accomplice to this utter perversion of the system on which we all depend for our scholarly recognition.

If Soviet scientists are to be denied the fruits of their honest labours, then Soviet science will soon be at the mercy of dishonest timeservers. You must be trembling inwardly with the thought that a higher patriotism than complete obedience to these orders would be to preserve for the Soviet Union the sceptical, critical, imaginative scientific way of life.

But my real question to you is—what should we do: what should I do, to help the scientific friend whom I have never met? I do not think I am greatly influenced in this by my own Jewish birth and upbringing, which in a free society I need neither exalt nor repudiate. A man in trouble has called on me by name, invoking the universalism of science, and the fellowship of our Invisible College, which knows no natural frontiers: I must surely heed his call.

One form of help is very easy to get in this case—publicity. All normal procedures of protest are being actively pursued, in the press, in government circles, through embassies, and so on. It is scarcely to be believed that much more can now be accomplished in this way.

Can anything be done through our scientific organizations and learned societies? It is my belief that this particular case—like a number of other cases in various countries (e.g. in recent years, Argentina and Czechoslovakia)—constitutes such an infringement of the norms of the scientific community that it is our duty to take notice of it officially. It seems to me, for example, that the agreements made between the Royal Society and the Soviet Academy of Sciences for scientific exchanges are based upon the assumption that these bodies have a similar respect for scholarly integrity, for scientific authority, and for the acknowledgment of scientific originality and priority. It would not, I believe, be a transgression of its principle of avoiding "politics" if the Royal Society were to question the official attitude of the Soviet Academy to these matters, and to indicate its unwillingness to cooperate with an organization that so openly failed to live up to the high ideals that we all profess to hold.

In the end, however, only a personal gesture is likely to be possible. That is why I am troubled about whether or not to accept your invitation to the Moscow conference. Should I refuse to take part myself in an activity sponsored by a government whose policy I detest, under the auspices of an organization—the Soviet Academy—whose hypocrisy I deplore? I realize that such action is largely symbolic. At some cost to myself (for it really would be pleasant and scientifically profitable to come) I cause a little damage to those whom I wish to influence and fail in my friendly duty to people, like yourself, whom I like and trust. Yet I think that the gesture is not quite useless, and that if it were copied by the many hundreds of British, European and American scientists who have been invited to scientific conferences in the Soviet Union this summer it might bring out very clearly the extent to which, as a community, we are united in detestation of these present actions by your government.

Tell me, then, colleague and friend, what I should do. I have never agreed with those who demand a boycott on visits to this or that country - Spain, Greece, South Africa, Czechoslovakia - on general political grounds. A scientific visit may look like support for the policy of the government in power: but in reality, it may be valuable moral backing for those men of integrity and good will who are struggling to establish scientific standards under very adverse circumstances. I prefer to put my trust in such men, and to accept their personal invitations in the name of scientific fellowship.

Is this what you had in mind when you sent me this invitation? Would I come as an official guest of your government, expected to speak in admiration of your scientific achievements and never to express, even in private, my inner feelings on this tragedy? Or do you ask me as a personal scientific friend, not only to discuss our common interest in certain technical questions of scientific research, but also to share our common concern for what is being done to another friend by this cruel world in which we all have to live? I am not speaking, of course, of reckless public acts of defiance, or any other such extravagant, useless gestures: merely of the spirit with which you would yourself meet me on this occasion. Until I know that, allow me to reserve my reply. I hope that you will still want me to come ; but I shall understand, and admire, a message to the contrary.

Yours sincerely,

JOHN ZIMAN

H. H. Wills Physics Laboratory,
University of Bristol

NEWS AND VIEWS

Solar Eclipse of a Lifetime, June 30, 1973

EVEN in the space age a total solar eclipse is a valuable tool in astrophysical and geophysical research. The longer the eclipse the more useful it is, and to attract ground-based scientists the Moon's shadow must cross some readily accessible land from which there must be a good chance of good visibility.

In the main, the solar eclipse tomorrow meets all these criteria. It is a very long eclipse with a peak totality of 7 min 4 s, which is not much less than the upper limit of 7.5 min, and which is considerably greater than the mean peak duration of 3 to 4 min. Not for several hundred eclipses, until June 25, 2150, will the duration of the forthcoming eclipse be exceeded. Peak totality will occur near where Niger, Mali and Algeria meet, which is not the most accessible of regions but, as the map shows, the shadow crosses more accessible countries, especially Mauritania and Kenya, where totality will last for not much less than the peak time. The chance of cloud cover is less than 30% over most of the African track, though deep in the Sahara atmospheric dust is likely to be a problem.

It is thus not surprising that the largest numbers of ground-based scientists will be in Mauritania and Kenya. Most of the French and Russians will be at Atar in Mauritania. Part of the US National Science Foundation (NSF) expedition will be at Chinguetti, Mauritania, and the rest of it will be at Loiyengalani, Kenya, except for a small group farther north at Koobi Fora at the edge of totality. At or near these principal sites there will also be several small groups from other countries, though a substantial number of Japanese will be at Eli Springs, on the opposite shore of Lake Rudolf from Loiyengalani.

Elsewhere, near the Cape Verde Islands, a Russian ship equipped with sounding rockets will be used as an observation platform, while just off the coast of Mauritania there will be a boatload of amateur astronomers from Britain. A few hardy groups will be strewn along the rest of the track, with a small concentration in Niger.

From the north of Mauritania an Aerobee rocket prepared by an Anglo-American group will be fired into the shadow, while coming in from the Canary islands will be Concorde 001 carrying two British experiments, two French experiments and an American one. Concorde 001 will fly along the eclipse path from Mauritania to Chad, thus extending totality to just under 80 min. Over the central part of this flight Concorde will be underflown by a group of American scientists in a USAF NC-135 aircraft, which will provide their ten or so experiments with 12 min of totality.

During a total solar eclipse it becomes possible to make Earth-based measurements that are otherwise difficult or impossible. Most of those planned are concerned with the solar atmosphere. This will be studied from the chromosphere, 1,500 km above the Sun's surface, out through the K corona (free electron scattering) to the F corona (dust scattering). There follows an indication of the range of solar measurements to be made on this atmosphere. No attempt has been made to be exhaustive. Finally, some of the experiments not associated with the solar atmosphere will be described.

The chromosphere is to have its optical spectrum recorded by a group from the University of Utrecht, and a group from Pennsylvania State University will study it at 3 mm and 8 mm to determine its temperature and density. Spicules in the chromosphere will be studied by a group from the High Altitude Observatory, Colorado. On board the Concorde a group from Queen Mary College, London, will examine the structure of the chromosphere by means of spectra obtained every 2 s between 0.3 mm and 1.5 mm. This experiment makes use of the 95% atmospheric transparency at these wavelengths at Concorde's 17,000 m altitude, and the slowed down motion of the Moon will result in improved height resolution of the chromosphere structure.

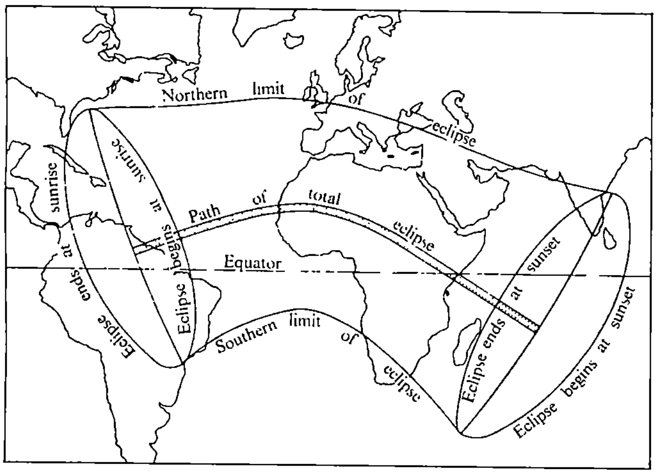
The chromosphere-corona transition is to be examined by the American Concorde team, from Los Alamos Scientific Laboratory (LASL). They will also look for waves in the lower corona, predicted to have a period of 300 s and which may "drive" the solar wind. The artificially extended totality will, of course, be a great advantage. A search for these waves will be made also by one of the French Concorde groups, from the Paris Astrophysics Institute, using a different technique.

The corona is the object of study of many of the experiments on board the NC-135. These originate from LASL and will involve spectroscopic, photometric and polarization studies at optical and near-infrared wavelengths. There are numerous ground-based corona experiments among which is a joint Franco-Russian effort to determine the time dependence of coronal jets by sequential observation from Mauritania and then Chad. This study is helped by the proximity of a sunspot minimum, which is associated with enhanced jet visibility. Various American groups will study coronal rotation, internal motion and solar latitude variations. A group from Sacramento Peak Observatory will try to detect certain broad ultraviolet absorption lines that have never been conclusively established.

The only ground-based rocket experiment has been prepared by a group from the Science Research Council at Culham and Imperial College, with rocket and support provided by Kitt Peak Observatory. The Aerobee rocket will reach a height of 150 km in the shadow and investigate the corona between 850 Å and 3,150 Å. This will increase knowledge of the structure, heating and magnetic activity of the corona and of any solar prominences that may occur. The solar prominences, like the weather, are capricious, but several ground-based groups also hope to investigate them.

The K corona gives way to the F corona at greater distances from the Sun. The K-F division is the subject of an experiment on the NC-135, and the F corona itself is to be studied by the other French Concorde group, from the Paris Observatory at Meudon, and also by various ground-based groups—for example a Franco-Russian group, and one from the University of Manchester which will be in Niger. The chief purpose of these experiments is to determine the spatial distribution of the F corona and composition of the dust grains comprising it.

There are a few experiments not concerned with the



Visibility of the total solar eclipse of June 30, 1973.

solar atmosphere. A group from the University of Texas will photograph the near-Sun star field to see if there are any distortions such as would be produced by gravitational bending of light grazing the Sun. A photographic search for new planets and comets near the Sun is to be made by a group from Dowling College. A few groups will be using the eclipse to make accurate determinations of the lunar diameter and orbit.

Finally, there are a small number of experiments associated with the Earth's atmosphere. The other British Concorde experiment will be conducted by a group from the University of Aberdeen who will observe at different heights in the Earth's atmosphere the decay of a metastable state of the oxygen molecule, normally sustained by solar radiation. An experiment on the NC-135 will examine forbidden emission lines arising from oxygen and nitrogen atoms in the upper atmosphere, while sounding rockets from the Soviet ship near the Cape Verde Islands will ascend to 180 km during the eclipse to record atmospheric density, ion composition, electron concentration and particle fluxes. On the ground a joint University of Botswana/University of Nairobi experiment will study the airglow, and a group from the University of Alaska will measure sky brightness. The relation between near ground atmospheric electricity and turbulence will be investigated by a group from the Naval Research Laboratory, Washington DC. A group from the University of Florida will investigate mechanisms of direct atmospheric solar heating by recording low frequency pressure waves, and a complementary group in Kenya from the Open University and Cornell University will carry out similar measurements.

This is clearly going to be an intensively studied eclipse. For those on the ground one must hope for clear skies.

B. W. J.

Dubious Mantle Plume

In the beginning was the Hawaiian chain, and the Hawaiian chain was the hotspot. The same was in the beginning with mantle plume. All things were made by mantle plume, and without mantle plume was not anything made that was made. Or so some earth scientists would appear to have us believe. But is the mantle plume the true light or does it, as R. T. Chamberlain said of Wegener's original continental drift hypothesis, take "considerable liberty with our globe, [being] less bound by restrictions or tied down by awkward, ugly facts than most of its rival theories"? Though far from being resolved, this is an important question because if hot spots and mantle plumes are proved to exist, they may, as Vogt is to suggest in a forthcoming article, lead to a unifying hypothesis for the earth sciences.

In the meantime, the process of sifting the evidence goes on, and papers supporting the idea of hotspots and plumes are becoming noticeably more frequent. Moreover, general theorizing is now giving way to more rigorous analyses of particular plumes. In recent months, for example, there have appeared three particularly convincing reports in which different types of data have been used to reach remarkable agreement on the areal influence of the supposed Icelandic plume. Thus Schilling (*Nature*, **242**, 565 ; 1973) has used rare earth and minor element geochemistry, Francis (*Earth planet. Sci. Lett.*, **18**, 119; 1973) has used seismicity, and Vogt and Johnson (*Earth planet. Sci. Lett.*, **18**, 49; 1973) have used both morphology and seismicity to show that there is a distinct limit some 900 km to the southwest of Iceland beyond which the nomal processes of seafloor spreading operate but within which the situation seems to be more complex.

On the other hand, those workers who have sought to use age data to plot plume traces across the lithosphere have been less convincing, partly because adequate sets of age data do not exist and partly, it must be said, because some ages are less consistent with the plume hypothesis than they might be. The only exception to this seems to be Clague and Dalrymple (*Earth planet, Sci. Lett.*, **17**, 411 ; 1973) who, notwithstanding serious gaps in the age data, are well on the way to making a good case for the applicability of the plum-hotspot hypothesis to the Hawaiian-Emperor chain. This is, of course, the region where it all started and where, some people would suggest, it ought to stay.

But in spite of the defects in the data, there is clearly something going on—and nowhere more so than in the vicinity of Iceland where conditions seem to be quite different from those elsewhere along the mid-Atlantic ridge system. Whether or not the differences are due to the effects of a rising mantle plume beneath Iceland is, of course, another matter ; opinions are divided and perhaps barely formed. There has been no shortage of vociferous opposition to the idea of mantle plumes, although most of it has been limited to conferences and university common rooms, and few opponents have been willing to commit their reservations to writing. In view of the sense of embarrassment still felt by many earth scientists (including those born long after the event) at the way that Wegener and his early supporters were vilified in the press by much of the geological establishment of the day, this wariness may not be surprising. It would be quite understandable if the memory of this unfortunate period of history were to induce a reluctance to commit to print opposition to an idea which could well turn out to sweep the board and thus subsequently make its opponents look foolish.

On the other hand, it would be unfortunate, for it is highly desirable that alternative explanations of the data should be brought forward and thoroughly tested. The article by O'Hara on page 507 of this issue of *Nature* is thus particularly welcome, for by seeking to account for the Icelandic condition without recourse to mantle plumes it represents one of the first serious attempts to show that data which have previously been taken to support plumes may well be explicable in more conventional terms. Thus O'Hara argues the case, rejected by Schilling, that the geochemical variations radiating from Iceland are caused not by the mixing of two primary magmas but by high and low pressure fractional crystallization dependent upon elevation. Presumably, the increasing elevation towards Iceland reflects greater magma flow but, as O'Hara admits, even his explanation begs the question of why this should be so. The point is, however, that if O'Hara's arguments are valid, the number of phenomena for which it is necessary to invoke a mantle plume is much reduced. P. J. S.

PROTEINS

Active by Association

from our Molecular Biology Correspondent

Sherlock Holmes's maxim that when you have eliminated the impossible, whatever remains, however improbable, must be the truth, has proved itself capable of wide application. It is implicit in the reasoning of Biswas and Paulus (*J. biol. Chem.*, **248**, 2894 ; 1973), whose studies on the subunit enzyme, aspartokinase, have brought to light a curious and so far unique situation. It is in all regards one of the more nightmarish allosteric systems, with control of activity by ligands operating apparently at three different sites, and, in addition, critical dependence of activity patterns on concentration and temperature.

The new work treats of the dependence of the functional properties on the subunit interaction. In the first place the stoichiometry has been established: in SDS-gel electrophoresis there are two subunits, α and β, with molecular weights of some 43,000 and 17,000, in equal molar proportions. The molecular weight of the native enzyme then corresponds to an $\alpha_2\beta_2$ structure. This Biswas and Paulus have confirmed by the invaluable cross-linking technique of Davies and Stark: treatment with a bifunctional imidoester leads to the appearance on SDS gels of four new components, in addition to the surviving monomeric α and β chains. They correspond in molecular weight to the species $\alpha\beta$, α_2, $\alpha_2\beta$ and $\alpha_2\beta_2$, thus confirming the tetrameric character of the enzyme. This distribution of cross-linked components has additional information content, however, for the absence of the β_2 or $\alpha\beta_2$ species must be taken to indicate that there are probably no β–β contacts in the native tetramer, which restricts the possible symmetrical arrangements to a form of sandwich structure, with an α–α core and one β chain attached to either α (but not to both, because then an $\alpha\beta_2$ species should be generated).

Separation of the α and β chains can be achieved without irreversible damage by dissociation to monomers in 4 M urea, and gel filtration. Removal of the urea from the α chains leads, as shown again by cross-linking, to a mixture of monomers, dimers and multiples of dimers, whereas the β chains form only some dimers.

Biswas and Paulus suggest that this pattern of self-association can follow simply from the sandwich-type structure of the tetramer, if the association sites are in some general sense adhesive, because then the α chains must be divalent, the stronger interaction occurring at the α–α interface, so that the dimeric unit is first formed, and the β chains univalent, and therefore capable only of forming closed dimers.

The interesting thing now is that not only is there activity in all the α chain species, but all the catalytic and allosteric characteristics are to be found in them. The total regain of activity, however, is no more than about 20%. No enzymatic or regulatory activity is to be discerned in the β chain. When now the renaturation experiment is done in an α–β mixture, the level of reactivation is increased by a factor of three, in a manner that depends on the square of the β chain concentration. The only role that Biswas and Paulus can envisage for the β chain therefore is as an aid to folding of the α subunits—and indeed, the very presence of β chains would of course be expected to create a trap for the native α conformation. It is, of course, conceivable that some other function may yet come to light, and also that the $\alpha\beta$ couple is the product of a single gene, and that a proteolytic scission occurs in the *Bacillus* cell after synthesis.

Immunotherapy of Cancer with Antibody in Rats

The complexity of anti-tumour immunity, where it exists, is taxing the minds of immunologists who seek to manipulate the immune response to favour the cancer patient. Experiments on animals have shown that T lymphocytes of thymic origin can be effector cells against tumour homografts and that unaided they can apparently kill the tumour. Similarly it has been found that macrophages armed with a product of lymphocytes which have been specifically stimulated can be cytotoxic for malignant cells. More recently it has become clear that a certain class of cell can kill tumour cells if they have been coated with the appropriate anti-tumour antibody but without this sensitizing antibody (LDA) no such lethal effect is evident. The protagonists of each of these killing systems are somewhat doctrinaire and each tends to think that his notions will be of the most use in the immunotherapy of cancer. Hersey, in *Nature New Biology* next Wednesday (July 4), argues the case for LDA and demonstrates that transfer of serum antibody *in vivo* can sometimes contribute to slowing down of tumour growth.

Hersey took a transplantable rat lymphoma which is syngeneic to the PVGc hooded strain of rats and used it to immunize xenogeneic (rabbit), allogeneic (A/gus rats) or syngeneic animals. The xenogeneic and allogeneic antisera were subsequently absorbed *in vivo* in non-tumour bearing PVGc rats to produce specifically anti-tumour antisera. The tumour cells injected into syngeneic recipients were irradiated heavily to prevent their growth. All sera were titrated for LDA activity using ^{51}Cr-labelled tumour target cells and human peripheral blood mononuclear cells as effector lymphocytes. Only the rabbit anti-tumour serum had any LDA activity after absorption. All the kinds of serum were then injected into PVGc rats before or at various times after implantation of the lymphoma. The survival times were studied and it was found that there was a significant prolongation of life in those animals receiving the absorbed rabbit antiserum. The effect was most marked if the serum was administered prophylactically.

Hersey considers various explanations of his results; for example, that the antiserum in conjunction with complement exerts a direct cytotoxic effect on the tumour cells or, alternatively, that the serum removes antigen–antibody complexes from the plasma of the recipient (by reaction with free combining sites on the antigenic component of the complexes) thus unblocking an effector pathway that was previously inhibited by the complexes. Inevitably he feels that the injected antiserum acts as an LDA *in vivo* as it had *in vitro*.

Hersey goes on to indicate that passive immunotherapy with heterologous antisera might be effective in situations in which LDA activity could be measured *in vitro*. They also seem to show that in a situation in which the host makes no immune response of its own—no anti-tumour antibody was produced in the syngeneic recipients of tumour—an artificial response can be contrived by using an antibody produced in another species of animal.

An interesting story concerning the role of subunit interactions in aldolase has been spun by Chan and his colleagues. They first showed that the activity of the tetrameric enzyme is simply that of an assemblage of identical monomers, by preparing tetrameric hybrids of native and inactive succinylated subunits. The also devised the subterfuge of coupling the enzyme to a column matrix at, on average, a single subunit, and then dissociating the tetramer so as to leave only isolated monomers bound to the column. These proved fully active, though relatively prone to denaturation. It thus seems that there are certainly two (and presumably also intermediate) active forms of aldolase. Chan *et al.* (*ibid.*, 2778) have now demonstrated that in the course of folding these coexist in the reaction mixture.

They have devised assays which will distinguish between the dissociated and tetrameric active forms. Discrimination is again achieved by the use of a critical urea concentration, in this case 2.3 M. In these conditions the tetramer is fully active, but the monomer unfolds. On the other hand, the instantaneous concentration of an active aldolase, monomeric and associated, in a mixture can be assayed by first introducing trypsin, which digests only the unfolded chains (or more precisely, because in optical terms refolding apparently precedes the appearance of enzymatic activity, the inactive population). Thus the kinetics of folding on transfer from concentrated guanidine hydrochloride into water can be followed by these two methods. The upshot is that total enzymatic activity appears according to a more or less first-order process, with no concentration dependence. In urea, by contrast, the kinetics show initially a curved profile, giving place to a lower linear rate, and are strongly concentration-dependent. The data give a convincing fit to a scheme whereby active monomers are first formed, and then associate to the urea-resistant tetrameric state. The identities of the urea-resistant and labile species were verified by determining the elution volumes of the two types of activity from a gel filtration column.

A striking artificial example of thermodynamic trapping of a native conformation by an association process is to be found in the work of Melchers and Messer (*Eur. J. Biochem.*, **35**, 380 ; 1973), who are engaged in sifting the details of the process whereby antibodies to active wild type β-galactosidase of *Escherichia coli* encompass the activation of practically inactive mutant forms. Evidently, the affinity of the antibody for the conformation characteristic of the active state is sufficient to tilt the equilibrium between the active and inactive structures heavily towards the former. The activation occurs on a time scale of minutes, and is associated with an activation energy of about 23 kcal/mole. There is no concentration dependence and evidently the conformational readjustment of the enzyme is the rate-limiting step.

Correction

In the editorial "Muscular Dystrophy and the Neurogenic Hypothesis" (*Nature*, **243**, 258, 1973) the authors of the letter on page 287 in that issue were given as Kümpel and Dubowitz. The reference should, of course, have been to Gallup and Dubowitz.

EVOLUTION

Crossing the Barrier

from our Plant Ecology Correspondent

As new principles and concepts develop within each of the principal biological disciplines, so there is a tendency to apply these new ideas to the well-worn questions relating to the early development of life on Earth. Palaeontologists, biochemists and cell biologists have already contributed much to knowledge of early living systems ; now it seems to be the turn of the ecologist to face some of these long-standing problems.

Stanley, for example, is an ecologist

Sorting Out Signals

Polypeptide chain initiation sites on messenger RNA can be isolated simply by forming test-tube initiation complexes with ribosomes and digesting away the unprotected regions of the RNA with ribonuclease. If the mRNA is appropriately labelled, the nucleotide sequence can be determined by any number of ingenious methods recently developed.

Hitherto such work has been confined to the small RNA viruses, Qβ, R17 and f2. Because DNA is not involved in the life cycle of these particular phage RNAs (unlike those of animal RNA viruses in general), the RNA must serve in other capacities besides that of directing protein synthesis: first, of course, the RNA must be replicated ; second, control of protein synthesis (both temporal and stoichiometric) can occur only at the level of translation ; third, folding of the phage RNA into the mature virus particle may introduce further constraints on the nucleotide sequences of RNA phage messengers in the regions of interest. The nucleotide sequences determining these various functions could conceivably overlap with the ribosome binding sites and be confused with polypeptide chain initiation signals.

Having pointed out the pitfalls in interpreting sequence data using the ribosome binding sites of RNA phage genomes alone, Arrande and Hindley announce in *Nature New Biology* next Wednesday (July 4) that they have now determined the sequence of the corresponding region of the polycistronic message (that which is transcribed by the host polymerase) for the "early" proteins of coliphage T7. The "early" genes are clustered at the left end of the T7 map and a single promoter site for the RNA polymerase has been identified by electron microscopy at the far left of this cluster. Arrande and Hindley have obtained a highly labelled "early" mRNA fragment of about 500 nucleotides by transcription of the phage DNA *in vitro* (a technique similar in principle to that used in their earlier work on R17 RNA). After digesting the labelled RNA-ribosome complex they were left with a piece of RNA thirty-eight nucleotides long whose sequence they analysed.

The only possible candidate (out of two) for the initiation triplet AUG for the first 9,000 molecular weight protein corresponding to gene 0.3 is that situated at positions 19 21 in the middle of the RNA fragment. This AUG is followed by the pentanucleotide sequence GAGGU which also occurs in the R17 A protein ribosome binding site. Moreover, the tetranucleotide GAGG appears in the corresponding region of the Qβ genome. The conservation of the sequence AUGGAGG (U) is consistent with the (logical) prediction that the ribosome recognition site involves more than the initiation triplet AUG and its availability in a hairpin loop in the messenger RNA. The initiation site has to be distinguished from the triplet for internal methionine residues which is also AUG.

Examination of many more ribosome binding sites, both those within polycistronic messengers, those of both cellular and viral DNA-coded messengers, and those of animal RNA viruses which introduce DNA copies into the cell by means of reverse transcriptase, will be necessary before such vital processes as "modulation" (differential rates of translation of individual cistrons in a polycistronic message achieved by the recognition of specific nucleotide sequences by different initiation factors and/or ribosomes), the piracy of the cellular protein synthesizing apparatus by infectious viruses, and other control mechanisms (for example, the choice between copying, replicating or translating RNA) can be explained (if not yet understood in terms of RNA-protein interactions). The fortunate nucleotide sequencers are going to have a field day sorting out the relevant signals.

who has been looking at the problems of why multicellular organisms arose suddenly at the close of the Precambrian and why the concomitant diversification of living organisms was so explosive (*Proc. Natn. Acad. Sci. U.S.A.*, **70**, 1486 ; 1973). This rapid radiation of life at the beginning of the Cambrian Period, which began about 570 million years ago, has formed something of a watershed in the geological record and is used as a marker to divide the Precambrian from the Palaeozoic. The rapidity of diversification (taking mere tens of millions of years) is all the more remarkable when it is realized that eukaryotic organisms had already been in existence for more than 1,000 million years, and the advent of sexuality, which might be expected to bring diversification, preceded this explosive radiation by about 250–300 million years.

Stanley suggests that the answer may lie in the trophic structure of these early communities. The relevant ecological principle which Stanley invokes is one formulated by the marine biologist Paine (*Am. Nat.*, **100**, 65 ; 1966) which states that the addition of a trophic level (that is a predator) to a community tends to promote diversity in the adjacent, lower trophic level (that is among the potential prey). The principle can be seen at work in many ecological systems ; the introduction of rabbit grazing to grassland increases plant diversity, whereas the removal of predation in some marine environments has been shown to reduce the diversity of benthic algae.

Stanley conceives of the late Precambrian world as an "all-producer" system, consisting largely of autotrophic, planktonic eukaryotes. This biological monotony could be relieved only by the development of cell-ingesting heterotrophs. This development itself would have been delayed by the slow rate of diversification in a system lacking any true trophic structure. The arrival of such heterotrophs (probably initially bacteria feeders) came in the late Precambrian, and the ability to ingest increasingly large particles would have led to the evolution of algal feeders. The crossing of this "heterotroph barrier", Stanley claims, could have led to the observed burst of diversification. The development of the carnivorous habit required little advancement and, once the food web had arisen, any diversification would lead to further diversification in adjacent trophic levels. Even the development of multicellular organisms could have received an important impetus from the arrival of heterotrophy.

Stanley's theory has much to recommend it because the principles he invokes, if not the system he considers, can be demonstrated experimentally. One important factor which it neglects is the build-up of oxygen in the Precambrian atmosphere as a result of the dominance of photosynthetic autotrophs. The coincidence of geochemical oxidative processes with the arrival of heterotrophy does suggest some causative interrelationship. Stanley's concept of the diversifying influence of heterotrophy in the Precambrian world may become even more plausible when considered in conjunction with contemporary changes in the early physical environment.

GRAVITATIONAL BIOLOGY

Living in Space

from a Correspondent

GRAVITATIONAL biology occupied only a very small part of the COSPAR programme at Konstanz (May 23 to June 6) and most of the contributions under this heading dealt with ground-based experiments using either centrifuges or klinostats. In the discussions it emerged that the unsatisfactory nomenclature used to describe the conditions of space flight can lead to disagreement as to the relevance of certain attempts at ground-based simulation. The factors necessary for the orientation of cellular processes as well as for the subjective assessment of the vertical need to be clarified. Water flotation, bed rest and forcible restraint in plaster have all been proposed as models for the reduced work load of free fall, and the confusion between "gravity" as a type of force field and "*g*" as a unit of acceleration leads to extrapolations from centrifuge studies to what has come to be spoken of as the "zero gravity" condition. Perhaps more attention should be paid to the density gradients produced by compression under contact forces rather than to effects attributable to the gravitational field itself.

Dr G. H. Bourne (Yerkes Primate Research Center, Atlanta), attempting to use bed rest as a model for the prolonged weightlessness of space flight, described histopathological changes in monkeys restrained for some months in plaster casts, the stress attributable to the restraint being alleviated by a well-planned social environment. The problems of bone repair during space flights were highlighted by Dr J. R. Beljan (University of California, Davis) who follows, with a collimated proton beam, the course of mineral deposition in a hole drilled through an avian long bone. Repair was much delayed after calcium

Cretaceous Normal Polarity Interval Defined

THE long period of normal geomagnetic polarity in the Cretaceous, discovered by Helsley and Steiner (*Earth planet. Sci. Lett.*, **5**, 325 ; 1968) and now usually referred to simply as the "Cretaceous normal polarity interval", is likely to prove a useful marker for worldwide correlation. It is all the more important therefore that not only should the interval be delineated as precisely as possible by the data available at any given time but that there should be agreement on its basic definition. Unfortunately, there is now a potential source of confusion in that two rather different definitions have been proposed or implied.

Helsley and Steiner did not offer a formal definition of the Cretaceous normal polarity interval but by referring to "this long period of normal polarity" implied a strict definition—that is, a period of single polarity without internal reversals, however short. From the continental palaeomagnetic results then available they judged the long normal period to stretch from the Upper Albian to the middle Santonian (about 25 million years from about 102 to 77 million years ago). McElhinny and Burek (*Nature*, **232**, 98 ; 1971), on the other hand, defined a rather longer interval (also from continental data) from 120 to 70 million years ago within which the field was predominantly normal but which included two known reversed periods at about 80 and 110 million years. Finally, Larson and Pitman (*Bull. geol. Soc. Am.*, **83**, 3645 ; 1972) used oceanic magnetic anomalies to give a completely normal interval between 111.5 and 84.5 million years ago.

In *Nature Physical Science* next Monday (July 2), Irving and Couillard propose that the Cretaceous Normal (Polarity) Interval as formally defined should refer to that period during which the field is completely normal ; in other words, they reject the basis of the definition by McElhinny and Burek. Of course, it is possible that having adopted the strict definition, short reversals within the Cretaceous Normal Interval may be discovered subsequently ; but Irving and Couillard argue that such a discovery need not "disturb the chronology". A new analysis of all continental data available up to the end of 1972 then gives 109–82 million years as the best determination of the Cretaceous Normal Interval. Because, however, this agrees so well with the oceanic determination by Larson and Pitman, Irving and Couillard propose limits of 110 and 83 million years as the best compromise currently possible, the uncertainties in these limits being ±3 million years.

depletion and feeding calcium supplements did not help. Dr L. R. Young (Massachusetts Institute of Technology), in a study of habituation, drew attention to an alternation of dominance between visual and vestibular cues in the perception of motion. Dr T. D. M. Roberts (University of Glasgow) showed with a mechanical model how his new scheme of labyrinth reflexes interact with the neck reflexes to stabilize the trunk, and suggested how the associated sensations might give rise to feelings of disorientation when astronauts move about freely in a large spacecraft.

The COSPAR meeting also provided an opportunity for further informal discussions on plans for the European Spacelab programme for 1980. The Council of Europe Working Party on Aerospace Physiology had recently been told that if it wished to propose a European experiment for any earlier NASA launch, it would have to be one which added no weight to the payload, occupied no space in the vehicle and consumed no power. Undeterred, Dr T. D. M. Roberts put forward a proposal which satisfies all these constraints, cannot be done on the ground, and promises a significant contribution to knowledge.

Information was to be collected on the interaction of neck reflexes with labyrinth reflexes in free fall conditions, using one freely floating astronaut as a subject, while another acts as a force generator, holding on to the spacecraft with one hand while with the other he applies linear accelerations in turn to the subject's head and to his trunk. Because the subject's arms are not engaged either in locomotion or in their own support against gravity, reflex movements should appear, and these should be visible from the ground over the existing television link. When this proposal was submitted in detail to NASA, yet a fourth insuperable constraint was revealed—the experiment must not occupy any additional astronaut time! A fully instrumented version of the experiment is to be included in the ESRO Spacelab programme.

NON-DESTRUCTIVE TESTING

Warsaw Meeting

from a Correspondent

AT the Seventh International Conference on Non-Destructive Testing, held at Warsaw from June 4 to 8, Drs P. D. Hanstead and R. C. Wyatt (Central Electricity Generating Board, Bristol) described how ultrasonic images of discontinuities in manufactured components can be visualized directly, without scanning, using relatively simple techniques. Pulsed ultrasound of a few MHz frequency, passing through a sample immersed in a liquid, is brought to a focus in a transparent medium, which is illuminated stroboscopically. Visualization is, in one instance, by the Schlieren method and, in the other, by photoelasticity. In the second case, where the transparent medium must be a solid, images formed by shear waves as well as by longitudinal waves can be observed.

Drs S. A. Lund and P. Jensen (Danish Welding Institute) dealt with the scanning of welds by ultrasonic shear waves, which enter the test sample obliquely, using what they call the "P scan", a modification of C scanning, in which a flaw indication is recorded in a position corresponding to that of the defect, yet independent of that of the transducer. They claim that the method provides an indication of weld defects with a definition better than that given by radiography. Dr H. Jünke (Ingenieurhochschule, Berlin-Wartenberg, DDR) gave an account of a method of testing safety glass using ultrasonics. A glass sheet immersed in water is scanned by Lamb waves, which are induced by mode conversion, and their group velocity measured. There is a decrease of about 6% in this velocity in "toughened" glass as compared with untreated glass.

In radiology, Drs K. F. Sinclair, H. A. Zagorites and K. A. Zimmerman (Xextex Inc., Belmont, California) described a method of scanning using a technique which they term "scintillography", in which no film is used. The signal from the received radiation is observed as a trace on a chart, which moves in synchronism with the examined object. The method can be used with X ray, γ ray, and neutron beam sources. Applica-

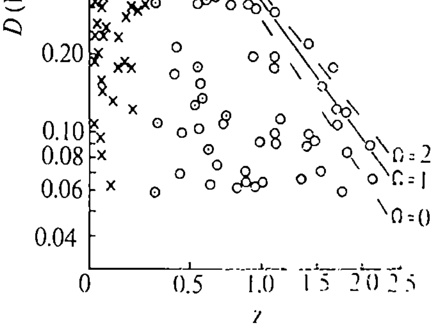
QSOs and Intergalactic Gas

IN *Nature Physical Science* next Monday (July 2) Strom compares observations of the radio polarization of quasars with measurements of their angular size, and suggests from the trends in the data that quasars are expanding into a significant density of intergalactic gas.

It is generally agreed that to obtain regular behaviour, quasars must be separated into those with flat radio spectrum and those with steep spectra. Flat spectrum quasars, believed to be recently exploded objects, show a wide scatter in strength, size and radio polarization, presumably because of random circumstances in their formation. The mature steep spectrum quasars, on the other hand, have angular sizes which decrease with increasing redshift z, as is expected, and furthermore show depolarization due to internal Faraday effects which increases at high z.

Different astronomers have put forward different explanations for this trend in the polarization data. According to Strom it follows naturally from the supposition that the quasars are expanding into an intergalactic gas. The density of the gas increases at great distances, as $(1+z)^3$, and decelerates the explosion, forcing the radio source to be contained within a volume which decreases with z. On average, therefore, the gaseous products of the explosion will have higher density, and will cause greater Faraday effects, if the quasar is at high z.

This hypothesis also succeeds in accounting for a curious feature of the angular size data. In a Euclidean universe, the angular size of an object decreases with redshift as z^{-1}. The geometry of a universe with relativistic cosmology is less simple, and for the most probable models the angular size stops decreasing and may actually increase again, at redshifts of about 0.25, well within the range covered by quasars. Miley's compilation (*Mon. Not. R. astr. Soc.*, **152**, 477; 1971) shows no signs of this "elbow" in the universe, and suggests that the steep spectrum quasars seem to follow the Euclidean law with no further cosmological factors. Such arguments assume all quasars to have the same intrinsic linear size. Strom's hypothesis, that their linear size varies according to the surrounding medium, though it cannot yet be put on a quantitative basis, does succeed qualitatively in removing a curious paradox.



Component separation, D, calculated on the basis of an Einstein-de Sitter ($\Omega=1$) metric and plotted against the redshift, z (the axes are log D and log $(1+z)$), for $D>60$ kpc. X, Radio galaxy; ⊙, quasar. Expected upper envelope for radio components decelerated by an intergalactic medium, $D \propto (1+z)^{-3}$.

tions include the testing of welds and the detection of foreign bodies in frozen food. In another radiological contribution, Dr G. C. Wheeler (General Electric Co., Schenectady) described a variable energy accelerator with an output of up to 25,000 r min^{-1} at 10 MeV, suitable for testing steel up to 20 inch thick.

A theoretical contribution on eddy current methods was given by Drs V. G. Gherasimov, V. V. Soukhorukov, and N. P. Rtishcheva (Power Engineering Institute, Moscow). They described an analogue process for simulating defects in ferromagnetic materials in order to predict crack depths very reliably. On the more immediately practical side, Drs W. A. Decker and D. L. Waidelich (University of Missouri) described the design of an electric field probe emitting short high energy pulses into dielectrics ; thus defects and metallic inclusions can be detected in plastic specimens up to 12 mm thick. A special feature is a correlation technique which, by making use of two receiving probes in different positions, keeps noise levels low.

A novel method of non-destructive testing was described by Dr H. Licht (Rheinisch-Westfälische Technische Hochschule, Aachen), in which Lamb waves are propagated in steel plates as a result of alternating Lorentz forces set up by eddy currents induced in the presence of a steady magnetic field. The transducer, in the form of a coil, does not have to be in contact with the material tested.

COSPAR

Plenty in Prospect

from a Correspondent

One of the highlights of the COSPAR meeting at Konstanz (May 23-June 6) was the series of contributions on the results from ERTS-1, the Earth Resources and Technology satellite. This satellite is in a 570-mile-high polar orbit and takes television pictures of 115-mile square regions of the Earth's surface in four colours—green, red and two in the near infrared. These pictures are proving of immense use for regional surveys of soil and terrain type, vegetation, geology, land use and water sedimentation and turbidity. The problems associated with mapping the more inaccessible regions of the world—for example, the Amazon Basin—are considerably lessened because the near orthographic view from ERTS-1 minimizes the distortion problems of conventional aerial mapping. Dr A. A. Jackson (University of Botswana, Lesotho and Swaziland) gave a dramatic account of how ERTS imagery is enabling Lesotho to make the best of its limited and scattered rainfall. In that country, which has few roads and limited communication, areas where rain had fallen and grass grown could easily be picked out from the images, thus enabling Lesotho to optimize its grazing potential and minimize the problems of overgrazing and subsequent soil erosion. Regions suitable for re-afforestation and diamond prospecting could also be identified.

Dr C. Sagan (Laboratory for Planetary Studies, Cornell University) revealed some of the latest information from the Mariner 9 mission to Mars. Dark streaks on the planetary surface were found to change with wind variations ; these streaks were caused by the scattering of light by the larger dust particles which are blown about more easily. Interestingly the data obtained from ground based observations of the dark markings on the planet give a perfect picture of the Martian wind and weather patterns during the past century. The sizes of the two satellites Phobos and Deimos have now been measured accurately; given in terms of the radii of spheres with the same area these are 5.7±0.5 km and 10.9±1.5 km respectively.

Problems of lunar chronology are still very much in evidence. Dr H. W. Hinners (NASA, Washington) questioned why the Moon, formed 4.6 aeons (4.6×10^9 yr) ago, should suffer many large impacts 3.9 aeons ago and then after considerable volcanism become relatively inactive 3.1 aeons ago. He also pointed out that the distinctive isotropic and chemical dissimilarities with Earth indicate that the Moon did not fission from Earth, the Earth–Moon system thus being the result of a dual planet formation or a capture mechanism.

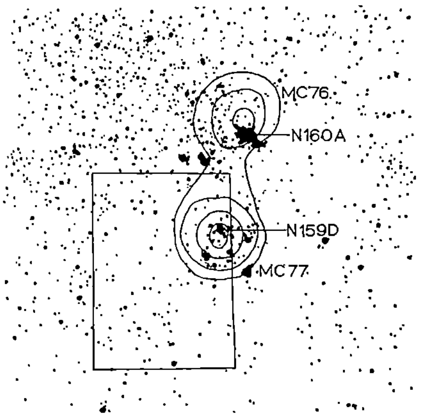
Returning to the Earth's atmosphere, Dr G. Witt (University of Stockholm) discussed the problems of deciding what forms the nucleation centres for noctilucent cloud crystals ; it is still not clear whether they are dust particles (blown up from below or incident from space) or large water molecules such as hydronium H_3O^+ $(H_3O)_2$. Dr M. Dubin (NASA, Washington) put forward the thesis that the blue haze seen sporadically on Mars is analogous to terrestrial noctilucent clouds and is formed by water condensing on accreted cosmic dust which is being blown out of the Solar System. The fact that the Sun is surrounded by a dust cloud is well established, but the density variation of this cloud is still a matter of considerable discussion. Preliminary results presented by Dr R. K. Soberman (Drexel University, Philadelphia) and Dr Matha S. Hanner (Dudley Observatory, Albany) from their meteoroid and zodiacal light detectors on Pioneer 10 indicate that this problem is nearing a solution.

Thinking of the future, Professor L. Biermann (Max Planck Institute, Munich) discussed the possibilities of sending space probes to intersect comets, the two candidates being comet Grigg-Skjellerup in 1976 and comet Encke in 1980. Slowing down the spacecraft to extend the period during which it is in close proximity to the comet is particularly important. Experiments to investigate the size and structure of the nucleus, to measure, using mass spectrometers, the chemical composition of the coma and to study the solar wind–comet interactions were all propounded. Dr S. I. Rasool (NASA, Goddard) led the conference on a trip round the Solar System by describing NASA's plans for future missions which include the July 1975 Viking spacecraft which will soft land on Mars and will, by analysing soil samples using a gas chromatograph–mass spectrometer, look for life on that planet. Another project discussed was the 1973–74 gravity-assisted mission to Venus and Mercury which will endeavour to take about 7,000 television pictures of Mercury to a resolution of 1 km and 5,000 during the Venus flyby. Equipment will also be carried to measure the surface temperatures, topographies and atmospheric compositions as well as the planetary masses, spins and radii.

Candidate for LMC X-1

The X-ray source in the Large Magellanic Cloud (2UO540-69) may be identified with the radio sources MC76 and MC77, according to Byrne and Butler (see *Nature Physical Science* next Monday, July 2).

Excluding 30 Dor, these two radio sources are the brightest in the LMC at 6 cm and 11 cm. Like five other known LMC sources, they form a double object with one thermal and one non-thermal component. They are identified with H II regions N 160 A (MC76) and N 159 D (MC77) and MC76 may be variable. At 55 kpc, the separation of the two sources is 120 pc.



Error box of LMC X-1 and 6 cm radio contours of MC76 and MC77 superimposed on B plate of the region.

Entropy and Demography

F. N. ARUMI

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A socio-physical theory describing the land distribution patterns of a system in demographic equilibrium is developed in this article.

THE behaviour of social systems, regardless of species, must conform to some natural laws in the same way as non-social systems. This article explores the feasibility of using equilibrium statistical theories in the description of large population aggregates. It describes the density distribution of land in a theory that can be formalized in terms of a disorder (entropy) function. The resultant distribution is a rigorous consequence of the postulates. As in statistical mechanics, the distribution is derived using general arguments that are quite independent of the mechanisms responsible for bringing about the equilibrium state.

In this approach it is the inhabited land that is distributed among the population. In order therefore to make discrete counting of land possible, I postulate the existence of a quantum of personal area. The rules for counting which I use assume the distinguishability of the population members and the indistinguishability of quanta. The total number of quanta is always greater than or equal to the population and I therefore postulate that all the inhabited land assigned to a given population is occupied.

A demographic system is defined here as an isolated, steady population residing in a given and fixed land area. A system in demographic isolation cannot exchange population or land with other demographic systems. The state of a demographic system is defined by specifying the total population and the total inhabited land. A configuration of the state is any possible distribution of the additional quanta among the population members. A microconfiguration in turn is any distinguishable arrangement of individual members of the population that yields a given configuration. A microstate is the generic term for all microconfigurations of a state regardless of the corresponding configuration. The total number of microstates is the sum of all the microconfigurations of all the possible configurations of a state. The disorder or entropy of a configuration or of a state is the natural logarithm of the number of microconfigurations or microstates respectively.

Number of Microstates

If the population and area of a state are given by N_0 and A_0 respectively, the number of additional quanta of area is given by

$$m=A_0/a-N_0 \tag{1}$$

where a is the magnitude of the quantum of area. The number of microstates, that is, the total number of ways in which m quanta can be distributed distinguishably among the N_0 individuals, is given by the combinatorial expression

$$\Omega=(m+N_0-1)!/m!(N_0-1)! \tag{2}$$

The disorder, or entropy, of the state is by definition

$$S=\ln\Omega \tag{3}$$

Expected Density Distribution

The total number of ways in which a population member may occupy quanta is given by the number of distinguishable ways there are of placing $(m+1-k)$ quanta into (N_0-1) individuals. The answer is given by the same combinatorial expression

$$g_k=(m+N_0-1-k)!/(m+1-k)!(N_0-2)! \quad (1\leq k\leq m+1) \tag{4}$$

Therefore, the fractional population in the kth level is

$$n_k/N_0=g_k/\Omega \quad (1\leq k\leq m+1)$$

where $\langle n_k\rangle$ is the expectation value of the population in the kth level. Using equation (2), this expectation value can be expressed as

$$\langle n_k\rangle=(m+N_0-1-k)!m!N_0(N_0-1)/(m+1-k)!(m+N_0-1)! \quad (1\leq k\leq m+1) \tag{5}$$

This expression satisfies the two basic conservation rules:

(i) conservation of individuals

$$N_0=\sum_{k=1}^{m+1}\langle n_k\rangle \tag{6}$$

(ii) conservation of area

$$A_0/a=m+N_0=\sum_{k=1}^{m+1}k\langle n_k\rangle \tag{7}$$

Table 1 illustrates the counting procedure leading to equations (2) and (4). Figure 1 illustrates the behaviour of equation (5).

Large Argument Approximations

For large arguments (N_0, $m\gg1$), equation (5), which is exact, can be approximated by a functional expression after using the relation

$$(n+x)!\simeq n!n^x \quad (n\gg1)$$

Thus

$$\langle n_k\rangle\simeq N_0^2/m(m/(m+N_0))^k \tag{8}$$

The behaviour of this approximate expression is compared with the exact results in Fig. 1. This result also satisfies the conservation rules provided that the upper limit of the sums is considered to be effectively infinite.

Theorem of Greatest Disorder

The expression for the entropy (equation (3)) can also be simplified. Applying Stirling's formula and then in succession equations (8), (6), (7) and (8), the expression for the entropy is recast into the form

$$S=N_0\ln N_0-\sum_{k=1}^{\infty}\langle n_k\rangle\ln\langle n_k\rangle \tag{9}$$

In this form, the distribution function (8) is readily recognized to correspond to the configuration that renders the entropy of the state an extremum.

Following the standard method of Lagrange multipliers to solve for the distribution that renders S an extremum, the demographic "temperature" of the system turns out to be

$$T=a/\ln\{(m+N_0)/m\} \tag{10}$$

and for the partition function of the microcanonical ensemble

$$f=1/\{\exp(\beta a)-1\} \tag{11}$$

where $\beta=1/T$. That a temperature function can be defined is a natural consequence of the use of equilibrium statistical arguments.

The behaviour of the average area per person ("internal" area) is displayed as a function of demographic temperature in Fig. 2. Real demographic systems, of course, are not in demographic equilibrium. This theory, however, is approx-

Table 1 Counting Procedures Leading to Equations (2) and (4)

State		kn_k^r = No. of cells to person					r or k	n_k^r = population in level k and configuration r					Ω^r	Ω	$g_k = \sum_r n_k^r \Omega^r N_0$				
N_0	m	1	2	3	4	5		1	2	3	4	5			1	2	3	4	5
3	1	2	1	1			1	2	1				3	3	2	1			
	3	4	1	1			1	2			1		3						
		3	2	1			2	1	1	1			6	10	4	3	2	1	
		2	2	2			3		3				1						
5	0	1	1	1	1	1	1	5					1	1	5				
	4	5	1	1	1	1	1	4				1	5						
		4	2	1	1	1	2	3	1		1		20						
		3	2	2	1	1	3	2	2	1			30	70	35	20	10	4	1
		3	3	1	1	1	4	3		2			10						
		2	2	2	2	1	5	1	4				5						

kn_k^r represents the number of cells a given person may have; in this case each person is assigned a number label, for example, for $N_0=3$, $m=3$ and configuration $r=2$, person 1 has three cells, person 2 has two cells and person 3 has one cell.

Ω^r = No. of ways configuration r occurs. Ω = No. of microstates $=\sum_r \Omega^r$. g_k = No. of ways a person may occupy k cells. Conservation of area: $m+N_0=\sum_k kn_k^r$. Conservation of people: $N_0=\sum_k n_k^r$.

imately applicable to systems in quasiequilibrium where the relaxation time is short compared with the typical time over which the ratio N_0/A_0 changes.

Empirical Test

In order to test the theoretical distribution I shall recast equation (8) into a form more readily matched with accessible empirical data. Cumulative population and area at level r are the respective sums of the population and area in all levels from $k=1$ to $k=r$.

$$N_r=\sum_{k=1}^{r} n_k=N_0\{1-(m/(m+N_0))^r\}$$

$$A_r=a\sum_{k=1}^{r} k\langle n_k\rangle=A_0\{1-[1+rN_0/(m+N_0)][m/(m+N_0)]^r\}$$

where N_r and A_r are the cumulative population and area respectively. An individual in the kth level has a personal area equal to ka. The population density in this level is therefore $p=1/ka$. Using equation (1) the cumulative area and population can be expressed as functions of density, depending on the total population (N_0) and the total area (A_0)

$$N(p)=N_0\{1-\exp(\beta/p)\} \tag{12}$$

$$A(p)=A_0\{1-(1+N_0/A_0p)\exp(-\beta/p)\} \tag{13}$$

where $\beta=1/T$, and the "temperature" T is given by equation (10).

I chose the urban population of the United States as the demographic system to be tested. As of 1960, the latest complete Census Data available at the start of this investigation, there were 5,409 towns listed with a population greater than 1,000 people[1]. This population is referred to here as the urban population. Smaller towns are not included because it was not possible to assign them a well defined area of occupation from the available information.

The demographic system is therefore comprised of the sum total of the population and area of these towns. The state parameters of the empirical system are

$$N_0=113{,}762{,}784 \text{ people}$$
$$A_0=33{,}887 \text{ mile}^2$$

and the density distribution ranges from 24 people mile^{-2} to 40,138 people mile^{-2}. The population, area and density of each town are then arranged in descending order of density. The empirical cumulative population and area are calculated by summing the individual values of population and area successively as the density scale is descended. The resulting values, plotted as a function of density, are shown in Fig. 3.

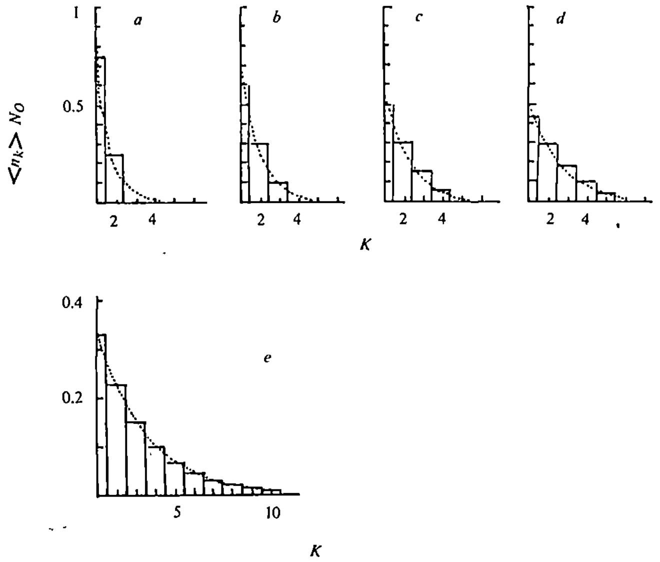

Fig. 1 Expectation value, $\langle n_k\rangle$, as a function of density level, k. —, Behaviour of the exact value, equation (5). ...., Analytical approximation (equation (8)). a, $m=1$; b, $m=2$; c, $m=3$; d, $m=4$ (all for $N_0=4$); e, $m=100$ ($N_0=50$).

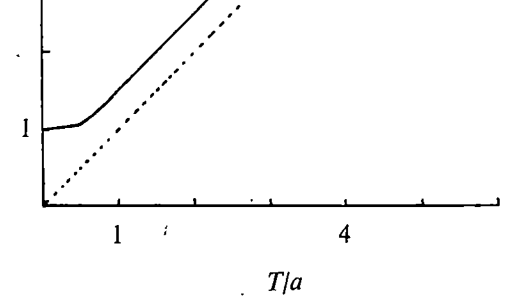

Fig. 2 The average ("internal") area per person ($A_0/N_0 \equiv u$) as a function of the demographic "temperature". For uncrowded systems, $A_0/N_0 \gg a$, the "temperature" is equal to the average area per person. As the "temperature" drops below a, the standing room only starts developing. See equations (13) and (1). Exp $(-a/T)=1-aN_0/A_0$; $u/a=(1-\exp(-a/T))^{-1}$. For $T/a \gg 1$, $u/a \simeq T/a+0.5$; for $a/T \gg 1$, $u/a \simeq 1$.

The theoretical distribution was generated by equations (12) and (13) using the corresponding state parameters.

The value of the quantum of area was used as a fitting parameter. It was found that no appreciable change in the theoretical predictions could be generated for values of $a < 620$ foot^2 (~ 62 m^2). The distribution obtained with values of $a < 620$ foot^2 yields the closest fit with empirical data. The validity of this upper limit is further reinforced by the fact that the highest density point in the data is less than 1/620 foot^2.

The theoretical distributions are shown as solid curves in Fig. 3. The agreement between empirical and theoretical density distribution of area is excellent. The agreement for the distribution of people is quite good although not as close as the one for area.

The disagreement may arise from a combination of facts. The demographic system studied is not truly isolated, as the 5,409 towns considered are in contact with the countryside and the data correspond to a period of rapid flux of rural population to urban sectors. The lower levels of population in the higher density regimes may be a consequence of the sprawling of American towns. The rate of sprawling may be higher than the relaxation rate of demographic systems seeking equilibrium. The system described is not in equilibrium. As such, a departure from the theoretical equilibrium is to be expected. Conceptually the theory remains ambiguous in the definition of inhabited land (A_0). A more precise definition will provide the theory with additional fine tuning capabilities.

Cross-cultural Validity

The formalism of the theory is independent of cultural, economic or political conditions of the demographic system. This implies that the same rules of land distribution should be detectable in any demographic system in the world. The predicted distribution therefore should be tested against empirical data from a variety of demographic systems that exhibit different cultural, economic and political conditions.

Demographic Contact and Clustering

A corollary of the theorem of greatest disorder states that the optimum entropy of two demographic systems brought into contact is always greater than or equal to the sum of the individual optimum entropies of the two separate systems. This means that clustering into single groups is more likely than staying as distinct and separate subgroups. This clustering tendency has been reported by behavioural scientists in their controlled observation of animals[2]. According to this theory therefore urban–suburban systems and twin cities should exhibit higher entropies than the sum of the component subsystems. This prediction can be tested by obtaining small grained data of well established cities with suburbs.

Limits of Growth

A system with standing room only value corresponds to a state where the number of quanta is equal to the population ($m=0$ in equation (1)). For a fixed land area, however, the entropy of the system is a maximum for a population equal to half of the "standing room only" value. If this is interpreted as being the occupationally most favourable size, then the maximum population would be equal to half of the "standing room only" value ($N_{\max}=A_0/2a$). Calhoun reports[3] that under controlled conditions, a mice population, whose standing room only value was to be 4,000, stopped growing at about 2,000. The agreement of this result with the prediction of this theory is remarkable. The relationship between the two uses of the expression "standing room only" value, however, remains to be determined. Verification of this phenomenon in human populations would provide an important test of the theory. Should it not be verified, however, it would not reflect negatively on the theory. Rather the interpretation of the population with the greatest entropy as the maximum population would have to be discarded.

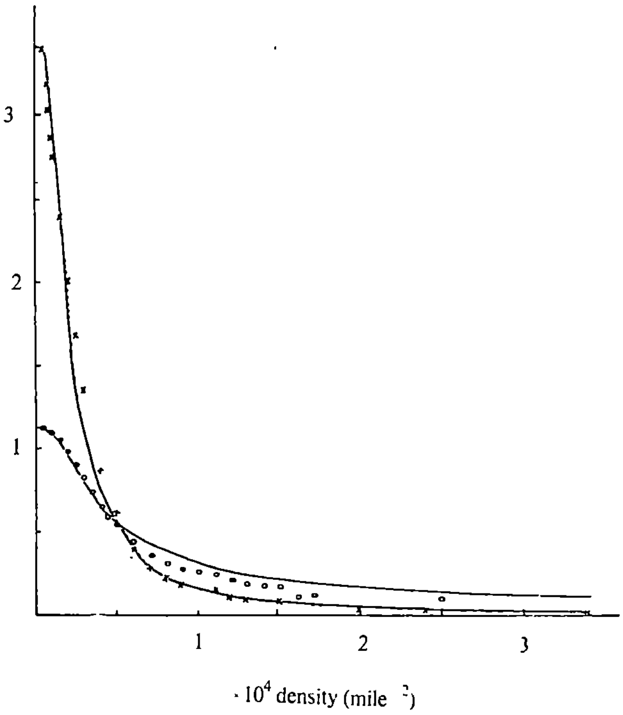

Fig. 3 Empirical area A_0 (10^4 mile^2) (×) and population N_0 (10^8 people) (○) as a function of density from the urban population data from the 1960 US census. Solid curves represent the theoretical predictions as generated by equations (13) and (12) respectively.

To carry out this test a demographic system with the following characteristics would have to be identified. (1) It would have to be demographically isolated; (2) there would have to be apparent stability of the population; and (3) the supply of food and shelter would have to be such that for the lack of these necessities would not be responsible levelling of the population.

Redistribution

This article has shown the potential usefulness of applying the methods of equilibrium statistical theories to the study of social systems. The theory was formalized in terms of a disorder function and the fundamental theorem states that a given population will redistribute itself within the inhabited land so as to maximize this function.

The predicted distribution was tested very successfully against empirical data taken from the urban population of the United States. The quantum of area was found to have an upper limit of about 650 foot^2. The possible relationship of this concept with that of personal space remains to be determined. More tests should be carried out to provide additional verification or to shed light on fundamental fallacies.

The internal density distribution of population in a single town should be compared with the theoretical prediction. This test may provide the means to identify more precisely the nature of the constraints of what constitutes the inhabited land area of a system.

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Reduction in the Concentration of Nerve Growth Factor in Mice after Sialectomy and Castration

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Surgical removal of the submaxillary glands in adult mice causes a profound drop in the plasma and tissue concentrations of nerve growth factor, and a reduction in the activity of tyrosine hydroxylase within sympathetic ganglia. NGF may be the protein which fulfils the retrograde flow of information from the axon terminal to the cell body.

THE recent development in this laboratory of a sensitive radioimmunoassay for the active subunit of mouse salivary gland nerve growth factor (NGF) has enabled detailed studies to be performed on the very low concentrations of this protein in tissues and plasma. Plasma concentrations of NGF in adult mice parallel the marked sex difference in NGF concentration in the submaxillary glands, and it was suggested that the normal tissue and plasma content of NGF is derived by secretion from the salivary glands[1,2].

In the studies described here, we found that surgical removal of the submaxillary glands in adult mice caused a profound drop in the plasma and tissue concentrations of NGF. There was, furthermore, a reduction in the activity of the enzyme tyrosine hydroxylase within sympathetic ganglia, which correlated with the reduction of the NGF content of the peripheral tissues innervated by these ganglia. On the basis of these findings a hypothesis is proposed concerning the trophic role of NGF in the sympathetic system.

Mice of the Swiss albino strain aged approximately 50 d were given Oxo diet 41B and water *ad libitum.* Submaxillary glands were removed under ether anaesthesia, the main vessels tied with silk ligatures and the wounds closed using Michel's clips. Sham operations consisted of freeing each lobe of the submaxillary gland and then closing the wound. The NGF was donated by Dr D. C. Edwards of the Wellcome Research Laboratories, and was a pure preparation of the β-subunit as described by Varon *et al.*[3]. For some experiments NGF was bound to aminocellulose powder as previously described[1].

Nerve growth factor was assayed by the previously described radioimmunoassay method[1]. Tyrosine hydroxylase was assayed by a radiochemical procedure involving the estimation of ^{3}H-L-dopa formed from side chain labelled ^{3}H-L-tyrosine[4].

For NGF assay, tissue samples were homogenized in 10 volumes of 0.05 M veronal buffer, *p*H 8.6, containing 0.9% sodium chloride, 0.5% bovine serum albumin, and 0.1% sodium azide. Blood samples for NGF assay were taken from the inferior vena cava of anaesthetized mice into a heparinized syringe, and diluted 1:3 (v/v) with the veronal buffer described above. Sympathetic ganglia were removed using a dissecting microscope; they were stored frozen (−20° C) in 10 µl of distilled water before the assay of tyrosine hydroxylase activity[4].

Submaxillary Gland and Plasma NGF

There was a marked reduction in the concentration of NGF in the plasma after the removal of the submaxillary glands in both male and female mice (Fig. 1). In male animals the fall in plasma concentrations of NGF had a half time of 9 d, and the concentration fell to a minimum of 15% of the normal adult control level by 33 d. After this there was a fairly rapid recovery to approximately normal plasma concentrations, which were reached by 60 d post-operatively.

In female animals the effects of sialectomy were qualitatively similar, with a half time for the fall in plasma concentrations of 6.5 d. The minimum value was reached earlier, however, and was not relatively as low as the male (approximately 25% of adult control values).

As normal male animals have a higher concentration of NGF in plasma than females, the minimum absolute values for plasma NGF after sialectomy were similar in males and females. The recovery phase in female mice was more prolonged than in males, plasma concentrations of NGF returning to normal only after 120 d.

This return to normal occurred in the absence of any significant regeneration of the NGF stores in the submaxillary or other salivary glands (Table 1). The concentration of NGF in the remaining salivary gland tissues (including parotid and sublingual) was slightly higher than that found in unoperated

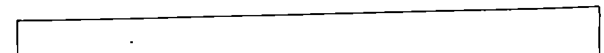

Fig. 1 Effects of removal of both submaxillary glands on the plasma concentrations of NGF in adult mice. Results expressed as means ± s.e.m. for groups of four–six mice. Control (100%) values were 2.6 ± 0.63 ng β-NGF ml^{-1} for female animals (O) and 11.5 ± 0.38 ng ml^{-1} for males (×).

Table 1 NGF Content of Mouse Salivary Glands in Normal and Sialectomized Adult Mice

Tissue	NGF (ng mg^{-1}) Female	Male
Control submaxillary gland	88±10.1	209±30.4
Control "residual salivary gland"	0.14±0.02	0.43±0.014
"Residual salivary gland" 17 d post-op.	1.15±0.16	0.50±0.03

The submaxillary gland was removed rapidly after death in the control animals, and 17 d before death in the sialectomized animals. The remaining salivary gland tissue (including parotid and sublingual glands) was assayed as a whole and classed as the "residual salivary gland".

animals. This may be attributable to some very slight regeneration of the submaxillary gland, or to the synthesis of NGF in granuloma tissue as reported by Levi-Montalcini and Angeletti[2].

Submaxillary Gland and NGF in Other Tissues

There was a gradual reduction of the NGF content of heart and superior cervical sympathetic ganglia in female mice after the removal of the submaxillary gland; but the fall in these tissues was slower than that observed in the plasma (Fig. 2). The NGF content of superior cervical ganglia fell to 50% of the control values after 15 d and the heart content reached 50% of the control concentration after 40 d. The lowest concentrations of NGF in these tissues were reached at a time when the serum concentration of NGF was already returning towards normal.

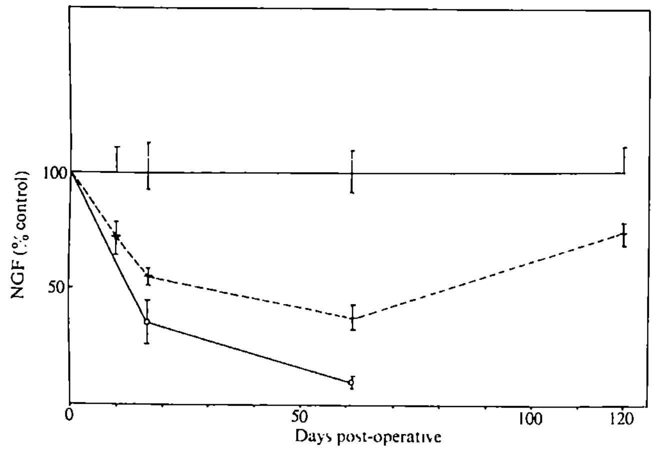

Fig. 2 Effects of removal of both submaxillary glands on NGF concentrations in the heart (+) and superior cervical ganglion (O) content of adult female mice. Values are means±s.e.m. for groups of six animals and are expressed as percentages of the control values (0.26±0.09 ng mg^{-1} for heart and 0.05±0.012 ng per ganglion).

Response of Sympathetic Ganglia

Tyrosine hydroxylase (T-OH) is an enzyme specifically located within adrenergic neurones in the sympathetic ganglion[5] and its activity in such ganglia is increased after administration of NGF to infant mice[4]. The activity of this enzyme in sympathetic ganglia from sialectomized animals was examined. A detailed study of the changes in T-OH activity in the superior cervical ganglia after the removal of the submaxillary glands was undertaken in female mice. A reduction in the activity of this enzyme was found within 10 d of the operation, and ganglionic enzyme activity reached a minimum of about 30% of normal adult values at 32 d, and slowly recovered to near normal levels by 120 d (Fig. 3).

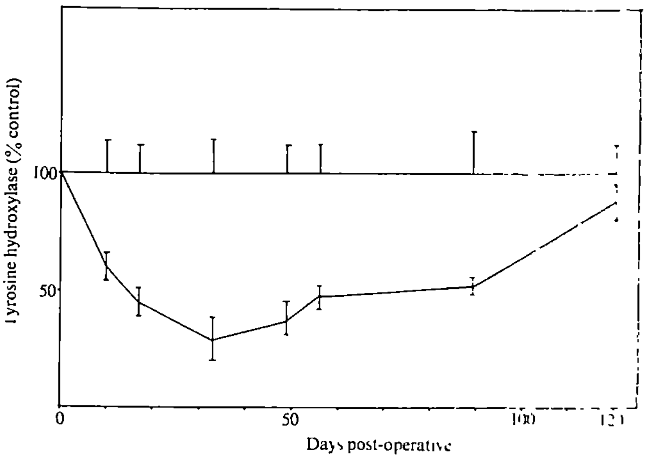

Fig. 3 Effect of the removal of both submaxillary glands on the total tyrosine hydroxylase content of superior cervical ganglia of adult female mice. Values represent means±s.e.m. for groups of four to six mice. Mean control value (100%) was 15.8±2.48 pmol dopa h^{-1} per ganglion.

In male animals there was also a fall in the T-OH activity of superior cervical ganglia after the removal of the submaxillary glands, but in this case the enzyme activity remained significantly depressed even at 56 d after sialectomy, when the plasma concentrations of NGF had returned to normal (Table 2). Thus, it seemed that the ganglionic T-OH activity might not depend directly on the plasma concentration of NGF, but corresponded better with the concentrations of NGF found in the tissues. The reduction in T-OH activity in superior cervical ganglia after the removal of the submaxillary glands may be caused by several factors. To determine whether the circulating concentrations of NGF, or contact of the adrenergic neurones in the ganglia with their end organs, which include salivary glands, would be the more important factor, the submaxillary gland was removed in some animals on one side only, and the contralateral side served as control. In these circumstances there was no reduction in plasma NGF; in fact there was a slight rise, probably as a result of damage inflicted on the intact gland during surgery (Table 2). The superior cervical ganglia on the operated side of such animals thus suffered from the removal of a major end organ but there was no change in the concentration of circulating NGF to which the ganglia were exposed. There was a fall in T-OH activity in the ganglia on the operated side, while the values in ganglia from the contralateral control side were unaffected (Table 2). Thus, the T-OH activity of the ganglia seems to depend more on contact with their end organ than on the circulating concentrations of NGF.

Table 2 Effects of Removal of the Submaxillary Gland and on the Concentration Plasma NGF and Superior Cervical Ganglion Tyrosine Hydroxylase Activity in Adult Male Mice

	Plasma NGF (ng ml^{-1})	Superior cervical ganglia tyrosine hydroxylase (p mol^{-1} per ganglion)
Sham operated	6.89±0.05	18.7±2.32
Left submaxillary gland removed		
17 d post-op.	11.80±2.50*	
Right side control		21.1±2.18
Left side operated		10.6±1.32†
Both submaxillary glands removed		
17 d post-op.	2.43±0.55†	8.9±0.99†
56 d post-op.	6.98±1.02	9.2±1.01†

Values represents means and standard errors for groups of six male mice. Plasma NGF concentration and tyrosine hydroxylase activity were assayed as described in the text.

* $P<0.01$.

† $P<0.001$ when compared with sham operated values.

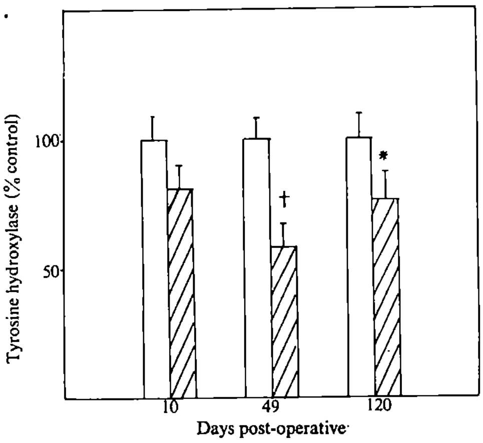

Fig. 4 Effects of removal of both submaxillary glands on the total tyrosine hydroxylase content of the stellate ganglion of adult female mice. Values are expressed as % of control tyrosine hydroxylase activity and standard errors (vertical bars) for groups of four to six animals. □, Control; ▨, operated. * $P<0.05$; † $P<0.005$ when compared with corresponding control values. Mean control value (100%) was 18±1.85 pmol dopa h^{-1} per ganglion.

Some of the adrenergic neurones in the superior cervical ganglia innervate the submaxillary glands, so the reduction in T-OH activity might be a result of retrograde degenerative effects in such neurones after axotomy. To test this, histological studies[6] of the ganglia were undertaken to determine if the fall in enzyme activity was associated with any damage to the ganglia or a loss of cells resulting from chromatolysis after axotomy. There was no significant change in the numbers of cells in the ganglia after sialectomy (Table 3), although there was a small but non-significant decrease in the average cell diameter.

Table 3 Cell Numbers in Superior Cervical Ganglia after Removal of the Submaxillary Gland

	Cell No.	Cell diameter (µm)
Control	12,913±588	14.9±0.77
Submaxillary gland removed	13,368±1,591	14.0±0.27

Effect of the removal of the submaxillary gland on the cell numbers and cell diameters of the superior cervical ganglion. The results express the means±s.e.m. for groups of six animals. Ganglia were fixed in 2.5% glutaraldehyde stained with cresyl violet and cell numbers and cell diameters estimated as previously described[6].

The effect of the removal of the submaxillary gland on the superior cervical sympathetic ganglion might be a result of the removal of the end organ *per se*, or of the removal of a trophic influence on the ganglion neurones, normally exerted in a retrograde fashion by the large amounts of NGF in the innervated submaxillary gland. The large reduction in T-OH activity found in the superior cervical ganglion after the removal of the submaxillary gland may reflect an intense effect of this type exerted by the very high concentrations of NGF in the mouse salivary gland. The relationship between T-OH activity and the NGF content of the end organ was accordingly examined in a different group of sympathetic neurones, those in the stellate ganglion. The postganglionic axons of the neurones in this ganglion should not be damaged by the removal of the salivary glands, and as they innervate the heart, this tissue was used to monitor changes in the end organ content of NGF as a representative sample of the tissues innervated by this ganglion. There was a fall in the T-OH activity of the stellate ganglion after sialectomy, and the extent of this fall correlated temporally with the fall in NGF concentration in the heart (Fig. 4). This finding lends further support to the view that end organ NGF may act as a retrograde trophic influence on the neurones in sympathetic ganglia.

Removal of Salivary Gland in Neonatal Mice

It was impossible to obtain an adequate survival rate in neonatal mice subjected to bilateral sialectomy. Animals more than 6 d old, however, survived unilateral submaxillary gland removal and grew normally. Animals operated at age 6 d and killed at 12 d showed a fall in the T-OH activity of the superior cervical ganglion on the operated side (Fig. 5). The decrease in enzyme activity was not as great as that observed in adult animals after unilateral sialectomy, and this may reflect the lower concentration of NGF in the neonatal submaxillary glands.

The role of NGF in the developing sympathetic nervous system is still unclear. Salivary glands of neonatal mice contain only very small amounts of NGF; it is possible that the major[1] source of NGF to the foetus derives from the maternal circulation. NGF labelled with ^{125}iodine is able to cross the placenta and is accumulated in the foetal tissues (unpublished observations).

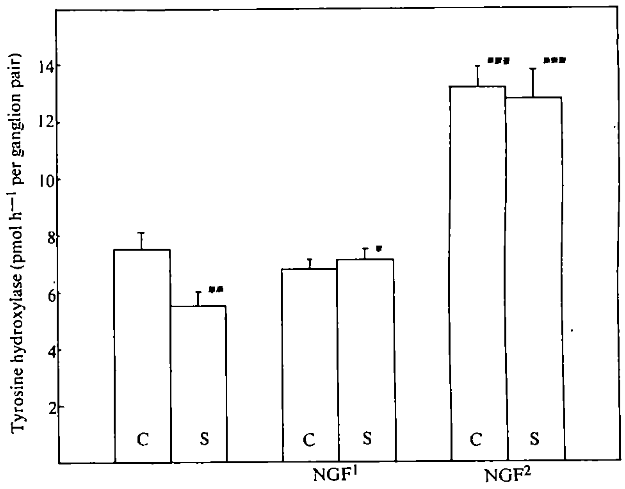

Fig. 5 Results of administration of NGF to neonatal mice after unilateral removal of submaxillary gland on tyrosine hydroxylase in superior cervical ganglia. Animals were operated at age 6 d and treated with daily injections of NGF as described in the text for 6 d post-operatively. Values represent means ±s.e.m for groups of six animals. Mean control value (100%) was 7.4±0.22 pmol dopa h^{-1} per ganglion. *c*, Value from control ganglion on non-operated side; S, value from ganglion on the side of removal of submaxillary gland; NGF^1, daily injections of solid phase NGF; NGF^2, daily injection mixture of solid phase and soluble NGF as described in text. * Does not differ from control but differs from sialectomized animals, $P<0.05$; ** differs from controls, $P<0.05$; *** differs from controls, $P<0.001$.

An attempt to examine the effects of lowering the maternal NGF concentration on the development of the sympathetic nervous system in the foetus was undertaken by removing the salivary glands in adult female mice. Unfortunately this operation produced a temporary infertility in these mice; in twenty-five animals none became pregnant within 100–120 d of the operation, by which time the maternal and foetal serum concentrations of NGF had returned to values not significantly different from normal.

In order to assess the physiological function of the NGF in animals in which the salivary gland had been removed, an attempt was made to replace NGF in the site of removal of the salivary gland. Unilateral submaxillary gland removal was performed in 6-d-old animals and they were subsequently treated daily with an injection of a suspension in saline of 2 µg β-NGF (bound by diazotization to 20 µg of cellulose) per gramme body weight into the site of removal of the submaxillary gland. The NGF-cellulose preparation released approximately 0.3% of the bound NGF d^{-1} and acted as a depot source of NGF. This injection of NGF prevented the reduction in ganglionic T-OH activity after the unilateral removal of the submaxillary gland. In order to assess the role of the localized action of the slow release of NGF compared with the effect of soluble NGF a similar experiment was performed using a mixture of soluble NGF and cellulose-bound NGF. A mixture of soluble NGF and cellulose-bound NGF was made so that 30% was bound to cellulose and 70% was soluble. The same total daily dose of NGF was given to the animals as in the previous experiment. In this case there was a marked stimulation of the tyrosine hydroxylase activity in both control and operated sides, but the difference in activity between these was again eliminated (Fig. 5). Thus, it would seem that the depot injections of NGF stimulated only the ganglion innervating the injected side, whereas soluble NGF had a more general stimulating action on sympathetic ganglia.

Castration, NGF and Tyrosine Hydroxylase

Thirty days after castration, NGF concentrations were markedly reduced in submaxillary glands, plasma and heart (Table 4). The fall in tissue NGF contents was again accompanied by a significant reduction in the T-OH activity of both cervical and stellate ganglia (Table 4).

Table 4 Effects of Castration on NGF and Tyrosine Hydroxylase in Mouse Tissues

Concentration of NGF	No.	Control	Castrated
Submaxillary glands	4	100±29.9	7.8±0.4†
Plasma	6	100±12.0	40.0±8.0‡
Heart	6	100±19.0	5.0±1.0‡
Tyrosine hydroxylase activity			
Superior cervical ganglion	6	100± 7.0	67.0±7.1‡
Stellate ganglion	6	100±11.0	69.0±7.2*

Animals were killed 30 d after castration, and the tissues assayed for NGF concentration and tyrosine hydroxylase activity as described in text. Value are means±s.e.m. for the number of animals indicated, and are expressed as per cent of control values from similar numbers of unoperated animals (for control values see Figs. 1–4 and Tables 1 and 2).

* $P<0.05$.

† $P<0.01$.

‡ $P<0.005$, when compared with corresponding control value.

Implications

Removal of the submaxillary glands from adult mice produced a reduction in the serum concentration of NGF in both sexes, indicating that the normal serum concentrations of NGF are dependent on the presence of intact submaxillary glands, which contain the principal stores of NGF in normal adult animals. After removal of the submaxillary glands, however, other as yet unidentified sources of NGF gradually emerged and were capable of maintaining normal serum concentrations. NGF increases in concentration in some fast growing mesodermal tissues, for example granulomas[2] and in the sarcomas 37 and 180 (ref. 7). The sources of NGF other than the submaxillary glands may reflect a generalized synthesis of NGF by mesodermal tissues rather than synthesis by any one particular alternative tissue. There was no marked increase in the NGF content of parotid or sublingual glands, nor in a variety of other glandular tissues after sialectomy. The alternative source tissues responsible for NGF production after sialectomy must, however, be responsive to male sex hormones, as the normal difference in the circulating concentrations of NGF in male and female animals was restored 2 months after sialectomy. The importance of circulating sex hormones in controlling NGF production offers another experimental means of altering tissue and plasma concentrations in adult animals. Castration also leads to profound reduction in the NGF concentration of mouse salivary glands, plasma and other tissues. This reduction in NGF production was again accompanied by a fall in T-OH activity in the superior cervical and stellate ganglia, although in this case the neurones in these ganglia did not suffer any surgical damage.

The regulation of the activity of the enzymes tyrosine hydroxylase and dopamine-β-hydroxylase in the superior cervical sympathetic ganglion and in the adrenal medulla of adult animals is partially attributable to trans-synaptic influences[8–12]. We have presented evidence here that the regulation of the activity of at least one of these biosynthetic enzymes, T-OH, may also depend upon the NGF content of the innervated end organ. There is a marked reduction in the activity of this enzyme in the superior cervical ganglion after the removal of the submaxillary gland, which may reflect the removal of a large pool of NGF from the field of innervation of that ganglion. The results of the experiments in which the T-OH activity of the stellate ganglion was compared with the NGF content of the heart after sialectomy seem to support this hypothesis.

The possibility of a retrograde flow of information from end organs to their innervating neurones has been under investigation for many years. In 1934 Hamburger[13] demonstrated an increased degeneration of motor cells in the developing chick central nervous system after limb amputation. Prestige[14–16] found a decrease in the number of cells in the dorsal root ganglion and in the ventral horn after amputation of the hind limb in *Xenopus*. In the brain, Cragg[17] has shown that lesions placed in specific areas may cause both orthograde and retrograde trans-synaptic effects.

A mechanism for the transmission of such information from the periphery has been postulated by La Vail and La Vail[18] who found that horseradish peroxidase injected into the optic tectum of chicks was transported in a retrograde manner to the cell bodies of the ganglion cells in the retina at a rate of about 72 mm d^{-1}. They suggested that this retrograde movement of protein from axon terminal to the cell body might represent a mechanism by which neurones responded to their target areas. The results presented here are consistent with such a hypothesis. In the sympathetic nervous system NGF may be the protein which fulfils this retrograde-information-transfer role.

The ability of NGF to reverse the effects of end organ removal on the T-OH activity of cells in sympathetic ganglia is consistent with the hypothesis that these effects are attributable to the removal of the trophic action of NGF. The different effects observed between the cellulose bound NGF and the soluble NGF are similar to effects observed by Levi-Montalcini and Hamburger[19,20], with different types of sarcoma growing in chick embryos. The small cell type of mouse sarcoma produced only low levels of NGF, and here only the sympathetic ganglia innervating the tumour were found to be enlarged. On the other hand, the large cell type of tumour had high NGF activity and these tumours caused a massive increase in all the sympathetic ganglia in the embryo.

The present hypothesis might also explain why adrenergic neurones in infant animals are more responsive to exogenously administered NGF than in adults. It would be expected that adrenergic neurones would be most sensitive to administered NGF during early stages of development, before their axons had established contact with NGF sources in their end organs. One might also postulate that during normal development only those adrenergic neurones which succeeded in establishing contact with their end organs would receive the supply of NGF needed for their continued survival. This could explain why the administration of NGF to young animals leads to increases in both the size and number of cells in sympathetic ganglia. The apparent hyperplasia evoked by NGF might represent in fact the artificially sustained survival of large numbers of adrenergic neurones that would normally have died through failure to make contact with their end organs.

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Salts in the Sea

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Salty hydrothermal solutions are discharged along rift valleys. Their composition gives insight into the chemical evolution of the sea.

THE first 10^9 yr of Earth history have left no stratigraphic record from which to decipher the chemistry of the primordial ocean. As a result, one can only speculate on its composition using criteria such as equilibrium models[1–3], geochemical balance calculations[4–9], or evolution of mineral facies in the Precambrian[10,11].

Here I use a new indicator for the old problem. My discussion is based on chemical analyses of hydrothermal fluids from the East African rift region, primarily Lake Kivu. I believe that these waters have received their salts under geological conditions similar to those that exist when waters are released to the sea along oceanic rifts and that this process was essentially the same during early stages in the development of the Earth's crust. Therefore, their chemistry will give us clues to the kind and quantity of salts that have been discharged from the oceanic lithosphere to the sea during geological times.

Geology and Hydrography

Lake Kivu is situated about 100 miles north of Lake Tanganyika, at the highest point of the East African rift valley 1,500 m above sea level. The greatest water depth measures 500 m; reducing conditions prevail below about 50 m.

The lake is surrounded by active volcanoes, and is fed by salty hydrothermal springs; stable isotope data indicate that the hydrothermal reservoir is charged by local rain water. This agrees with sedimentological studies of a 12 m core section of the past 25,000 yr; periods of hydrothermal activities and heightened volcanism seem to coincide with pluvial times[12–14]. These rain waters, enriched with volcanic CO_2, accumulate their salt load by percolating through the rift rocks at elevated temperatures, and are finally discharged into the deep lake as thermal springs.

An examination of the lake's structure reveals a unique stratification pattern. Temperature and salinity increase in a stepwise manner with depth (Fig. 1). Such steplike profiles, observable both in nature and the laboratory, are a result of convection[15]. The individual water layers or cells are well mixed within themselves and separated by sharp boundaries. The development of such water cells permits retention of dense hydrothermal fluids in the deepest part of the lake. As long as a certain flux of heat and salt is maintained, the layers will stay intact with little or no mixing with surface waters. Calculations on the residence time of the salts, using sodium as a reference standard, yield an age of 350 yr.

Below the thermo and halocline (>50 to 60 m), the analyses indicate linear relationships among most elements. The *p*H of the water is 9.2 at the surface and <6 at depths

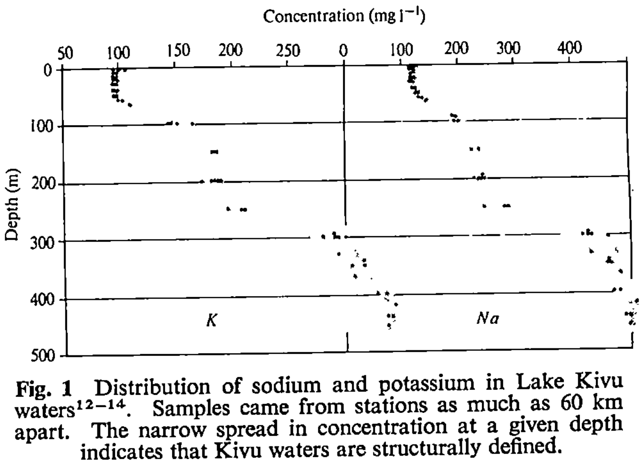

Fig. 1 Distribution of sodium and potassium in Lake Kivu waters[12–14]. Samples came from stations as much as 60 km apart. The narrow spread in concentration at a given depth indicates that Kivu waters are structurally defined.

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Table 1 Water Analyses of Lake Kiyu

Depth (m)	Ca	Mg	K	Na	Cl	SO_4	ΣCO_2	P	Si	$N(NH_4)$	$N(NO_3)$	N(urea)
	mg l^{-1}								μg atmos l^{-1}			
0	5	87	97	122	55	24	572	1	231	18	2	0.4
100	64	147	145	192	86	25	1,280	19	424	487	0.4	2
200	83	182	179	245	110	166	1,850	33	428	1,314	0.3	2
350	111	394	315	465	205	214	5,850	53	1,056	5,460	0.5	5
440	113	417	338	487	215	220	7,040	55	1,226	7,105	0.3	3

Table 2 Water Analyses of Kabuno Bay

Depth (m)	Ca	Mg	K	Na	Zn	F	ΣCO_2	P	Si	$N(NH_4)$	$N(NO_3)$
	mg l^{-1}								μg atmos l^{-1}		
5	35	97	117	125	<0.5	1.4	n.d.*	0.5	65	3	0.2
20	67	355	281	283	1.4	int‡	n.d.	1	589	30	0.1
40	127	478	336	363	2.1	2.6	n.d.	11	1,636	461	0.2
120	92	803	416	471	4.5	2.3	6,420†	66	2,122	552	0.1
140	91	823	427	481	7.2	1.9	7,130	70	2,240	565	0.1

* Not determined. † Measured at 130 m. ‡ Iron hydroxide interference; 55 mg Fe l^{-1}.

greater than 250 m. A representative profile from the centre of the lake is presented in Table 1. Kivu water below 300 m will release ~1.5 l of CO_2 and 0.4 l methane l^{-1} water at NTP. Because concentrations of CO_2 and methane are below saturation at depth, these gases are also trapped by the strong stratification pattern of the lake. Other gases detected include nitrogen, hydrogen sulphide, a few light hydrocarbons, and possibly hydrogen.

Sodium and chlorine levels in the deep water are definitely controlled by hydrothermal emission; however, the extent of hydrothermal control for other elements was not discernible at first. For example, magnesium and calcium may precipitate as carbonates; heavy metals can be removed as sulphides close to the point of hydrothermal discharge; and effects of microbial activity on CO_2, SO_4, CH_4, PO_4, NH_3 and trace metal concentrations are only poorly understood. Within a few hundred years many reactions might indeed proceed. Data from an expedition to Lake Kivu in March 1972 added more conclusive information.

In 1948 a lava flow reached the northern shore of Lake Kivu and dammed off a portion of the lake 15 km long, 8 km wide, and reaching 150 m in depth. This part, known as Kabuno Bay, is presently connected to the main Kivu basin by a narrow, shallow channel. Since this incident, hydrothermal waters accumulated and almost completely filled the bay. The chemical analyses of some ions and nutrients in the bay water are shown in Table 2. CO_2 and sodium values are the same as those in deep Kivu basin water. The other elements show only moderate differences between the two sets of analyses.

I think that the chemical composition of deep Lake Kivu and Kabuno Bay water roughly mirrors, in kind and proportion, the original salt composition of hydrothermal solutions emanating from active rift zones. Based on this assumption, the type and quantity of salts released to the ocean via hydrothermal channels can be calculated.

Hydrothermal Salt Flux

To estimate the oceanic hydrothermal salt flux, a major ion must be found which is derived principally from hydrothermal processes rather than from weathering of crustal material, and is not appreciably lost from the oceanic system by processes such as precipitation or evaporation. Chlorine fits the description best. On the basis of geochemical balance calculations[16], only 0.5% of chlorine in the oceans actually comes from the weathering of igneous rocks.

In Table 3 (column *A*) the concentrations of salts in Lake Kivu have been adjusted for a chlorinity of 1.935%. The ΣCO_2 is included in this Table to emphasize the abundance of free CO_2 in the hydrothermal system; this amount certainly varies from hot spot to hot spot. Converting the major cations present in the sea to oxides would yield a value of ~5 kg cm^{-2} of the Earth's total surface which would be equivalent to a sediment layer about 20 m thick over the whole Earth[17]. A corresponding calculation for an ocean filled with hydrothermal water (Table 3, column *A*) will yield a shell around the Earth of ~260 m.

Calculations for some trace constituents enriched in Kivu waters are also presented in Table 3. The value for iron has been corrected for sulphide formation close to the point of hydrothermal discharge. Converting the trace metal ions to oxides, these oxides could cover the Earth's surface in a layer 20 m thick.

Table 3 Determination of Hydrothermal Salt Flux

	Ocean*	Kivu	*A*	*B*
	g kg^{-1}			
Na	10.76	0.48	44.23	66.35
K	0.39	0.43	39.62	59.43
Ca	0.41	0.09	8.29	12.44
Mg	1.29	0.82	75.56	113.34
Cl	19.35	0.21	19.35	29.03
SO_4	2.71	0.22	20.27	30.41
HCO_3	0.72	7.13†	657†	986†
	μg kg^{-1}		g kg^{-1}	
Si	4	63	5.80	8.7
P	0.07	2.1	0.19	0.29
N	0.5	7.9	0.73	1.10
F	1.4	2.3	0.21	0.32
Fe	<0.1	40	3.69	5.54
Mn	<0.01	1	0.09	0.14
Zn	<0.01	7.2	0.66	0.99
Cu	<0.005	0.2	0.02	0.03

* To obtain the total mass (kg) of each element in the ocean multiply the numbers in this column by 1.37×10^{18}.

† Expressed as ΣCO_2.

$A = \text{Conc (Kivu)} \times \frac{\text{(Cl ocean)}}{\text{(Cl Kivu)}}$

$B = 1.5\ A$.

Sinks for hydrothermal chlorine include recent and ancient sediments as well as crystalline rocks derived from metamorphism and granitization of former sediments. Chlorine fixed in sediments in the form of interstitial waters, salt deposits and ordinary minerals constitutes about 25% of the amount present in the sea[5,8,16]; excess of chlorine in granites, diorites, syenites and metamorphic rocks above the amount released from weathered basalts yields another 25%. The sum total of hydrothermal chlorine is thus about 50% above the present seawater chlorinity. Column *B* in Table 2 has been adjusted for these contributions; recalculating the thickness of the oxide shell around the globe, a figure of slightly more than 400 m is obtained of which heavy metals comprise 30 m. Alternatively, if all hydrothermal metals were extracted in the form of oxides at a constant rate for the Earth's entire history, this rate would be $\sim 10^{-5}$ cm yr^{-1}.

Chemical Evolution of Seawater

Assuming that chlorine was added to the sea as a salt rather than as a volatile constituent, and that the remaining ions in the hydrothermal solutions generated along rift zones have an approximate Kivu-type distribution, then initially seawater was magnesium and carbonate rich. Over geological time seawater evolved to sodium chloride dominance; but how did this occur?

In following the concept of Sillén[17], I propose that reactions between the solutes in the sea and the minerals in sediments are responsible for the steady state conditions. Work on deep sea sediment cores[18] indeed indicates a marked decrease of magnesium and potassium from interstitial solutions and a corresponding increase in calcium; however, interstitial chloride and sodium concentrations remain constant. Potassium may be incorporated into illites and glauconites, and magnesium may enter into clay minerals such as chlorite and montmorillonite. Magnesium may also be removed as sulphate and carbonate.

Interactions between seawater and minerals frequently lead to liberation of hydrogen and calcium ions, in exchange for potassium, iron and magnesium ions[1-4]. The increased availability of calcium in turn promotes a more effective extraction of magnesium in the form of dolomite and high magnesium calcite, and eventually results in massive limestone production. In this manner the initially large reservoir of free CO_2 can be gradually depleted until the carbonate equilibrium which presently exists in the ocean is established. A fraction of the carbon dioxide is fixed as reduced carbon by inorganic or biological means. Carbon isotope data indicate that—at least since the Cambrian—about one-third of the incoming hydrothermal CO_2 is removed in the form of reduced (=organic) carbon and two-thirds in the form of carbonates[19].

Sulphate can be deposited as magnesium and calcium sulphate. With the advance of anaerobic bacteria capable of reducing sulphate, sulphides provide an even more effective sink.

Geological data support this line of reasoning: clay minerals in Precambrian and early Palaeozoic shales are chiefly chlorites and illites, while kaolinite and expandable clays are more abundant in younger sediments[20]; Ca-Mg carbonates show compositional changes with age starting with high magnesium members in the Precambrian and developing towards almost pure $CaCO_3$ in the Cretaceous[21,22]; carbonaceous chondrites—considered to have originated in a primitive hydrosphere—contain the low temperature minerals magnesium-iron chlorites, epsomite ($MgSO_4 \cdot 7H_2O$), limonite, ferroan magnesite (breunnerite) and gypsum[23]; and there is a lack of rock salt deposits in strata of Precambrian age.

Kivu sediments contain many of the mineral phases of early rocks[12-14]. If they are subjected to diagenesis, banded magnesium-iron silicates, iron oxides, siderite, dolomite, sulphides and chert deposits will develop, closely resembling Precambrian iron formations.

Flux of Interstitial Water

Recent sediments contain on average 90% water by weight (96% by volume). The amount of water in ancient sediments varies between 10 and 50% by weight; this variability is determined by texture, structure, composition, age and compaction history of the rock. Seawater is thus constantly lost to the sediment column[24]. By calculating the mean annual sedimentation rate, the average thickness of sediment material (compacted to zero % H_2O) which accumulates in 100 m.y. in the ocean basins is around 650 m; expressed in terms of a sediment layer around the entire Earth's surface, a value of 460 m is obtained. Assuming that these rates have been fairly constant throughout the Earth's history, a sediment mantle, about 20 km thick, would cover the Earth. This sediment mass would be about twice the mass of the present continental crust.

In the process of mountain building, sediments become folded, metamorphosed, and granitized. Sedimentary water is expelled and is transformed into hydrothermal fluids. These fluids carry certain ions and gases away from the rock system, and eventually return, after many transformations, to their starting place, the ocean.

In order to calculate the quantity of water involved in orogenetic events, I assume that at the time of complete or partial melting a sediment still holds about 50% water by volume. Dividing the interstitial water volume by the volume of water contained in the world ocean—with a mean ocean depth of 3,729 m (ref. 25)—about 8 times the volume of the present oceans has passed through the continental crust and oceanic trenches, that is, one complete cycle takes 500 m.y. In contrast, rivers renew the ocean every 50,000 yr.

Assuming that marine sediments were the primary source of sedimentary and crystalline rocks composing the continental crust, it follows that weathering and erosion simply recycle ancient marine deposits. The most significant factor controlling the concentration of marine salts is seawater–mineral interaction which determines the rate at which individual ions are removed from the hydrosphere either syngenetically, diagenetically, or orogenetically. Because of the long residence time of meteoric water in geosynclines and the continental crust, and the elevated temperatures to which geosynclinal sediments are commonly subjected, it seems to me that seawater is presently close to equilibrium with continental rocks. The position of equilibrium will shift gradually in response to the input of new salts from rift activities. As the mass of continents increased with time, the significance of hydrothermal rift salt contributions on seawater composition grew less and less.

Overall Model

Two types of hydrothermal water can be recognized. The first is associated with active rift zones, and the second is generated from geosynclinal sediments in the process of mountain building. Both are principally of meteoric origin. Ascending rift water supplies new salts to the ocean, whereas rising geosynclinal water chiefly recycles salts derived from ancient sediments. A Soxhlet-extraction analogy adequately describes the principles involved. A rising pulse of hydrothermal water reacts with oceanic and continental rocks picking up salts and gases. Some of this dissolved materal is removed from the fluids in response to pressure, temperature or chemical changes. The remainder of the solution is released to the hydrosphere and atmosphere, thereby bringing the cycle to a close. Thus, the composition of the Earth's atmosphere and hydrosphere is interrelated with rift and geosynclinal activities.

The enrichment of sodium and potassium in the continental crust relative to the oceanic crust is attributable to the addition of alkalis to the ocean in the vicinity of rifts, the removal of potassium by clays, and the involvement of sodium-rich formation water during granitization. The sources of hydrothermal ore deposits are heavy metals and sulphur previously concentrated by sedimentary processes. Further, hydrothermal wall rock alteration may explain some of the mineral and rock assemblages observed in rift zones (for example, greenstones and greenschists).

Seawater began as a carbonate solution rich in magnesium. It gradually evolved to a sodium chloride dominance by a series of mineral equilibria. The excess in CO$_2$ was removed by carbonate deposition and inorganic and biological CO$_2$ reduction. Steady state conditions were probably established at the end of the Precambrian, at which time organisms began to form a carbonate shell.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Non-Primary Magmas and Dubious Mantle Plume beneath Iceland

Basalt lavas dredged from the mid-Atlantic ridge south of Iceland exhibit a conspicuous correlation of minor element chemistry with distance from Iceland[1]. This also happens to be a correlation with altitude, those lavas which contain the higher concentration of incompatible elements (and the higher Fe/Mg and Na/Ca ratios) being collected from greater heights above the general level of the ocean floor. The lavas are predominantly quartz-normative and most contain phenocrysts of olivine, augite and plagioclase.

Schilling[1] has rejected differing degrees of fractional crystallization as an explanation of these relationships. He prefers a process of mixing of two primary magmas, the one generated by partial melting of depleted upper mantle spreading from the ridge, and the other generated by partial melting of undepleted deeper mantle rising as a plume beneath Iceland.

Here I summarize the evidence that these lavas are not primary magmas, but may have suffered extensive low and high pressure fractional crystallization. I re-examine the proposition that a varying degree of fractional crystallization is responsible for the variation in lava chemistry and offer an alternative interpretation of the observations, without recourse to mantle plumes, in terms of a hypothesis of magma genesis at the upper mantle-transition zone boundary.

Experimental petrology of quartz-normative basalts[2] has shown that they are not in equilibrium with olivine at pressures greater than 2 to 5 kbar. They cannot, therefore, be primary magmas derived from a peridotite upper mantle at greater pressures because olivine (and enstatite) will be residual crystalline phases in that material when the partial melting occurred. Olivine and calcic plagioclase are, moreover, not stable together at pressures greater than 8 to 10 kbar (ref. 3) so the phenocryst assemblage cannot be explained as residual from the source mantle. This point has been made previously with respect to ocean floor tholeiite[4] when I pointed out that olivine would not be present at the liquidus of the more siliceous of such rocks beyond ~2 kbar or the more basic beyond ~7 kbar. This has been confirmed recently by experiment[5] and enstatite does not appear at the liquidus of these rocks at any pressure (the pigeonite which appears transiently in one sample investigated by Kushiro and Thompson has an Mg/(Fe+Mg)=0.845, much more iron-rich than presently suggested for residual upper mantle mineral compositions).

The values of M (=100 Mg/(Mg+Fe) in the lavas change in such a way on passing northwards along the ridge that they would be in equilibrium with an olivine with M=83 to 78 (if all iron is present as FeO) or 86 to 81.5 (if 20% of all iron is present as Fe$_2$O$_3$) assuming the Roeder–Emslie[6] empirical relationship to hold. These indicated olivines are approximately twice as ferriferous as the olivines of postulated fertile upper mantle (M=88 to 92) in the case of iron-rich basalts allegedly derived from the undepleted mantle plume, and also iron-rich relative to the olivines of postulated[7] depleted upper mantle (M=96 to 94). In simple terms, these basalts are too rich in iron relative to magnesium to represent primary magmas from likely upper mantle compositions and an interval of olivine fractionation is indicated in order to decrease their M values relative to the true primary magmas[4].

A true primary magma derived by substantial partial melting at pressures of 10 to 25 kbar as suggested by Schilling (ref. 1, Fig. 4) should be at a temperature above its liquidus on arrival at low pressure and, moreover, its composition should bear no special relationship to that of a liquid in simultaneous cotectic equilibrium with several crystalline phases at low pressures. Yet it is clear that most of the basalts under discussion were erupted with phenocrysts of olivine, augite and plagioclase present. They seem to have had the temperature and composition of liquids in cotectic equilibrium with these three crystal species at low pressures and this has been confirmed experimentally in the case of the 1783 Lakagigar fissure eruption[8] which seems to belong to the same petrographic group. Such relationships are a mandatory consequence if low pressure fractional crystallization had modified the magma compositions.

Nevertheless, basaltic liquids can be in equilibrium with olivine, augite and plagioclase over a considerable temperature range. It would be expected that those lavas erupted at the higher levels, which have higher Na/Ca and Fe/Mg ratios and higher Ti, K, P and REE than those erupted at lower levels, would have the lower liquidus temperatures and must, therefore, have been erupted at lower temperatures in order to contain the observed phenocrysts. This can easily be verified by experiment. Such a relationship accords with a model in which greater amounts of low pressure fractional crystallization affect those magmas which have to rise to higher levels before eruption. The temperature relationship is the reverse of that predicted by the plume model (ref. 1, Fig. 4).

The way in which the erupted magmas might be related to the true primary magmas has been explored from the theoretical point of view[2,4] and substantiated by studies of an actual province[9]. Once olivine, augite and plagioclase are fractionating together at low pressure, or when eclogite fractionates at high pressure, large reductions in the residual liquid volume are possible without dramatic changes in the character of the residual magma, except with respect to its concentration of incompatible elements. The basalts under discussion seem to have been erupted from the condition of olivine-augite-plagioclase fractionation, but the basalts richest in the rare earth elements do not show the relative depletion in the lighter rare earth elements which is expected when significant fractionation of clinopyroxene has occurred.

The $(\mathrm{La/Yb})_{\mathrm{EF}}$ ratio in the basalts poor in incompatible elements and collected south of 61° N (ref. 1, Table 1) is 0.28, indicating very substantial enrichment of heavy REE in these magmas relative to chondritic ratios. In the basalts rich in incompatible elements and collected north of 63° 50′ N, the $(\mathrm{La/Yb})_{\mathrm{EF}}$ ratio is 2.04, indicating massive relative and even absolute depletion of the heavy rare earth elements simultaneous with an approximately four-fold increase in the light REE.

Light REE depletion in the more primitive looking, potassium and titanium-poor basalts from the southern part of the ridge may be ascribed either to clinopyroxene fractionation, or better to derivation from the partial melting of a garnet-harzburgite source rock, which would represent a partially residual mantle[4,7] after the prior elimination of clinopyroxene from the assemblage. Only eclogite fractionation from such liquids appears capable of producing residual liquids with the extraordinary depletion of heavy REE, simultaneous with massive enrichment of other incompatible elements which the more northerly basalts display.

I now suggest that all these basalt magmas erupted between 60° N and 65° N on the mid-Atlantic ridge may be derived from a similar parental magma. The differences arise because the outflow of magma was persistently greater in the area of Iceland itself[10] leading to a greater elevation of the superstructure above the ocean floor. Magmas rising through this thicker superstructure exert a higher hydrostatic pressure at the source region and take much longer periods between formation and the eruption of some of the magmas on the surface, allowing greater scope for fractional crystallization at all pressures during ascent. The remarkable abundance of acid differentiates in Iceland fits naturally into this model.

The relative enrichment of incompatible elements, such as K, P, Y, implies that those basalts erupted at the highest levels (on Iceland itself) represent the residual liquid after the crystallization as olivine-gabbros or eclogites of at least 75% of some parental liquid if fractional crystallization is the sole cause of the variations.

The ultimate source of the primary magmas might be postulated to be the base of the upper mantle, which is the most likely region for an intersection of the geotherm with the solidus. Such a process, which requires a substantial heat flow from the lower mantle, has side effects capable of explaining plate decoupling, ocean floor spreading, "convective" return within the upper mantle and the geochemical problems of magmatic provinces[11]. It does not require the extensive flow of solid mantle which is required by the "plume" or simple convection hypothesis, but I concede that it is no more successful than any other interpretation at explaining why Iceland should be a point of enhanced magma flow.

The grounds on which Schilling[1] rejected fractional crystallization as the origin of the geochemical differences between the abyssal and subaerial lavas were the increased ^{87}Sr/^{86}Sr and ^{207}Pb/^{206}Pb values in those basalts enriched in incompatible elements. The assumption that these isotopes cannot be fractionated is implicit. In the face of the evidence for substantial low and high pressure fractional crystallization of these basalts, it may be necessary to re-examine the proposition that isotope ratios do not change during such a process. At least part of the geochemical differences commented on by Schilling[1] are due to low pressure fractional crystallization. Only after these effects have been carefully unravelled will it be possible to determine whether or not there is any residual evidence for mixing of magmas from two fundamentally different mantle sources, one of which might then be interpreted as a mantle plume.

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Wave-like Disturbances in the Ionosphere

BEER[1] has recently suggested that atmospheric gravity waves could be expected to exist in the ionosphere as a result of the supersonic motion of the terminator. He reached this conclusion by drawing an analogy between the proposed production of gravity waves by the supersonic motion of the Moon's shadow on the Earth's atmosphere during a solar eclipse[2], and the supersonic motion of the Earth's terminator. At the mesopause the extent of the terminator's supersonic motion can be as great as ±55° latitude.

We find Beer's suggestion particularly interesting in the light of our observations of the behaviour of ionosphere electron temperature and density measured by the Langmuir probe experiment on ESRO-1A (refs. 3 and 4). During the lifetime of the satellite the rotation of the orbital plane with respect to the Earth–Sun line, and the precession of the position of perigree in the orbital plane, meant that a sweep of local times was obtained at all latitudes and over a range of altitudes. Plots of electron temperature and density against UT were prepared for each orbit and visual inspection of these plots revealed on occasions the wave-like disturbances first reported by Raitt[5], and described here. Careful checking of satellite "house-keeping" data indicated that the observed variations were of geophysical origin, and were not due to equipment malfunction or varying satellite attitude.

Useful data were retrieved from 2,200 of the first 2,800 orbits of ESRO-1A, and these data were searched for the occurrence of wave-like disturbances. Evidence of such disturbances was found on 120 orbits. All 120 examples occurred after the satellite entered the dawn sector and were usually restricted to the latitude range ±60°.

An example of the effect is shown in Fig. 1 which shows the variation of electron temperature around a complete orbit. The electron density always showed a similar variation, although with a rather complex phase relationship that could not be clearly established. For illustration purposes we have used the linear scale temperature plot because the wave-like nature of the disturbance is more pronounced than in the logarithmic density plots. (An additional feature of Fig. 1 is the occurrence of "sub-auroral temperature peaks"—narrow bands of greatly enhanced temperature occurring at conjugate latitudes between about 45° and 55° during times of magnetic disturbance[6].)

The waves detected by ESRO-1A first became evident in early February 1969 when the local solar times for the satellite equator crossings were 0700 and 1900. They were still evident in late April, when the equator crossing times were 0500 and 1700; that is, the waves appeared to be restricted to a band within 2 h (30° longitude) of the terminator. The example of Fig. 1 shows waves only in the dawn sector; although on a few occasions of spectacular disturbance the waves also extended into the dusk sector where they were of very much smaller amplitude. Because of the configuration of the orbit it was impossible to determine whether this small amplitude revealed a true difference in dawn and dusk behaviour, or an altitude dependence.

If the horizontal wave velocity is very much smaller than the satellite velocity, then a "wavelength" estimate may be obtained by multiplying the time between successive maxima by the horizontal component of satellite velocity. For the 120 examples detected by ESRO-1A, this resulted in a distribution of "wavelengths" which peaked near 1,000 km.

The fluctuations we observe may be magneto-hydrodynamic waves stimulated by gravity waves in the lower ionosphere causing plasma motion parallel to the magnetic field, but able to propagate only in those regions of the ionosphere where Lundquist's criterion $BL\sigma\rho^{-\frac{1}{2}} \gg 1$ is valid, where B=magnetic field, L=characteristic size of plasma, σ=conductivity of plasma, ρ=density of plasma.

This would go some way to explaining the asymmetry in our results between dawn and dusk for which the orbit configuration is such that the satellite is generally at higher altitudes in the dawn sector than the dusk sector; also even at similar heights the plasma conditions at dawn are quite different than at dusk. In fact both situations tend to satisfy the Lundquist condition since in the dusk sector we have a dense (O^+) ionosphere of lower conductivity than the high less dense dawn sector of ($H^+/H_e{}^+$) ionosphere of higher conductivity.

Because of the restricted times (within a few hours of the terminator position) and locations (within the latitude range ±60°) of the ESRO-1A observations of wave-like disturbances, we put forward our observations as possible evidence for Beer's hypothesis that gravity waves could be generated at the terminator longitude and would be expected to produce ionospheric effects as a result of the supersonic motion of the terminator.

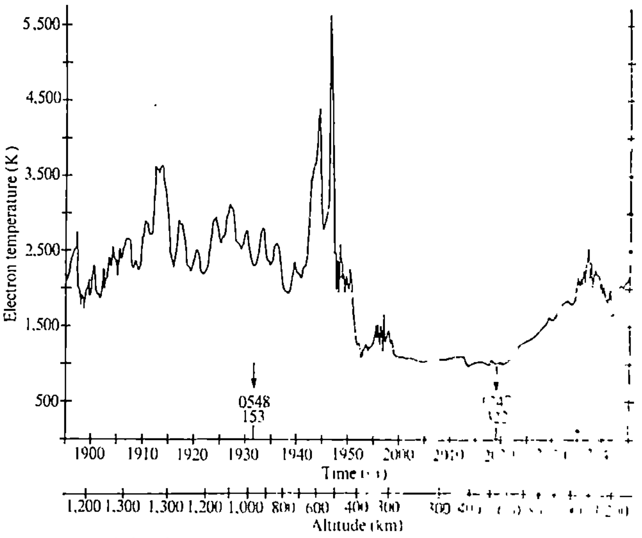

Fig. 1 ESRO-1A electron temperature data. Orbit 2,604, April 5, 1969. Arrows (↓) mark LST of equator crossing (top) and longitude of equator crossing (bottom).

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Phosphate in Sediments off South-western Africa

Recent work[1] has shown that the unconsolidated sediments of the continental shelf off south-western Africa are considerably enriched in phosphate compared with the values considered usual for clastic sediments (<0.2% P_2O_5), and it is interesting that phosphorite still appears to be forming on a significant scale in the diatomaceous muds off south-west Africa[2,3]. This may be the first well documented occurrence of modern phosphorite formation.

As part of a general geological survey we have determined the distribution of phosphate in 900 samples collected on a 10-mile grid extending from the coast to depths of 1,500–2,000 m (Fig. 1), allowing us to present a more detailed picture than hitherto possible of the distribution of phosphate and to provide a regional synthesis of the origin of the deposits. The phosphate determinations were made colorimetrically by the Phosphate Development Corporation, Phalaborwa, Transvaal.

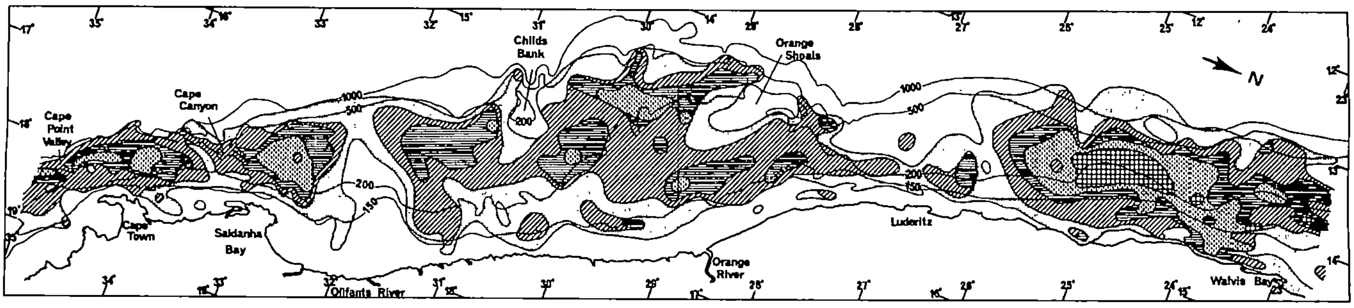

Fig. 1 The distribution of phosphate (as % P_2O_5) in unconsolidated surface sediments from the continental margin of south-western Africa.

□, 0 to 0.5%; ░, 0.5 to 1.0%; ▨, 1.0 to 2.5%;
▤, 2.5 to 5.0%; ▥, 5.0 to 15.0% ▦, >15% P_2O_5

The bathymetry is based on soundings by the Marine Geology Group, University of Cape Town, and depths are in m.

We find that the phosphatic sediments (>0.5% P_2O_5) are concentrated on the outer continental shelf (150–500 m) and that they differ in character to the north and south of Lüderitz. To the south they contain, on average, 2% P_2O_5 and at most 9% P_2O_5. The sediments richest in phosphate (>2.5% P_2O_5) are concentrated on the outer shelf (200–500 m), except between Lüderitz and the Orange River where the concentration is chiefly on the middle shelf (150–250 m). The sediments of the inner shelf (<150 m) and those of the slope (>500 m) are not commonly phosphatic, except south of 32° S where phosphatic sediment is found as deep as 1,000 m.

The phosphatic sediments of this southern area are chiefly sands and muddy sands, some of which are gravelly and many of which are glauconitic, the glauconite concentration reaching 90% (ref. 4). This mineral is associated with subangular, sand-sized fragments of phosphorite, and may itself be partially phosphatized[5]. The fragments of phosphorite are identical to the large slabs of this rock commonly dredged from the middle and outer shelf in this region[6]. These slabs are assumed to be fragments of Eocene to Miocene bedrock, much of which is glauconitic[7,8]. We consider that the phosphatic and glauconitic sediment associated with the phosphorite is probably a residual or lag-type placer deposit mantling a downwarped late Tertiary erosion surface[4,5]. The preservation, at surface, of these largely relict sediments is due to the very slow rate of late Tertiary and Quaternary sedimentation prevailing over the outer shelf. Where this rate increases, as on the inner shelf and on the slope, the relict phosphorite is either buried or diluted.

Low phosphate concentrations on the outer shelf reflect slight variations in the sedimentation pattern. For example, off Lüderitz it seems that highly quartzose sediment from the Orange River was widely distributed during low sea level times, thus burying or diluting residual phosphatic sediment[9]. On the Orange Shoals and the Childs Bank, Quaternary carbonate sedimentation has been slightly higher than on the deeper surrounding seafloor and the relict phosphatic components of the sediment on these rises has been diluted correspondingly. By contrast, the low phosphate content of the sediment off the Olifants River is due to burial of the downwarped Tertiary erosion surface by a prograding wedge of terrigenous muds. The phosphate depletion in the sediments of the Cape Canyon and Cape Point Valley, which cut through Neogene and Palaeogene strata and locally exposed Cretaceous rocks[7,8], may reflect their derivation from older (Palaeogene or Cretaceous) and less phosphatic outcrops than those beneath the middle and outer shelf. An isolated sample of phosphatic sediment (5.4% P_2O_5) from the canyon floor represents transported material.

North of Lüderitz, phosphatic sediment (>0.5% P_2O_5) covers much of the inner shelf and uppermost slope in addition to the outer shelf. The phosphatic sediment of this northern area contains, on average, 4% P_2O_5 and, at most, 22% P_2O_5. Near Walvis Bay, diatomaceous mud is widespread in the inner shelf (50–150 m). Our samples of this mud usually contain <0.5% P_2O_5 both at surface and at depth within sediment cores up to 1 m long, and the distribution of the mud is clearly reflected by that of phosphate-poor sediment (see Fig. 1). Richly phosphatic sands, which may be gravelly or muddy but are not normally glauconitic, occur on the outer shelf seawards of the ooze. The phosphate of the sands is concentrated in well-rounded, dark brown (ferruginous and manganiferous), sand-sized pellets (with median diameter of 0.1–0.5 mm) of more or less pure phosphorite. The phosphate mineral is francolite, and the pellets contain about 32% P_2O_5. One mechanism of pellet formation has been proposed by Soviet geologists[1-3] who found, scattered within the diatomaceous muds, some soft, friable, sand and gravel-sized clots of phosphatized sediment. They suggested that these clots would lithify with further diagenesis and that subsequent mechanical reworking of the mud during changes of sea level would give rise to concentrations of pellets and small nodules of phosphorite. Some of the pelletal deposits so formed have been subsequently lithified in the past to form pelletal conglomeratic phosphorite. We have dredged angular fragments of this rock type, containing up to 20% P_2O_5, from the centre of the zone of richly phosphatic pelletal sediment between Lüderitz and Walvis Bay. As some of the pellets in the overlying sediment are clearly composite, erosion of this outcropping phosphorite must also have been a source of pellets. The relation between the apparently modern phosphorite of the ooze belt, the unconsolidated phosphatic pelletal sands of the outer shelf, and the pelletal conglomeratic phosphorite is evidently very complex and a detailed coring and dredging programme is required to resolve it.

Both to the north and south of Lüderitz many of the largely terrigenous fine-grained sediments of the inner shelf and upper slope are very slightly phosphatic (0.2–0.5% P_2O_5). These same sediments are relatively rich in organic matter (>1% C_{org}) and contain abundant faecal pellets. The enrichment of the sediments in organic matter seems to have given rise to conditions favourable to the diagenetic formation of small amounts of phosphate minerals in such microenvironments as faecal pellets and foram tests[1,6]. The main exceptions to this pattern are on the inner shelf off the Orange River, where the rate of sedimentation of terrigenous detritus is very high, and on the lower parts of the slope at depths where organic matter is very scarce because of its solution in the water column[10]. The only other major source of phosphate is fish debris, which was mainly concentrated, although in very small amounts, in the relict sediment of the outer shelf[1].

The abundance of phosphatic sediment on this shelf is indirectly due to the upwelling of nutrient enriched oceanic waters and consequent high biological productivity. Modern phosphorite seems to be forming diagenetically in the fine-grained organic rich sediment typical of this area of upwelling. But it is only forming in very small amounts. The bulk of the phosphate is bound in relict detrital gains of phosphorite. These were derived in the late Tertiary, or during low sea level times in the Pleistocene, by mechanical reworking of diatomaceous muds (north of Walvis Bay) or by the erosion of previously existing phosphorite (throughout the area). The lack of widespread modern phosphorite formation signifies a wide difference in environmental conditions between the present time and the early to middle Tertiary (when phosphorite formation predominated locally).

The rich relict phosphate deposit near Walvis Bay is probably about 0.5 m thick and may constitute a reserve of some 4×10^9 t P_2O_5. The grade of the deposit could be beneficiated by screening and its potential lies in its situation 700 miles nearer to Walvis Bay than the nearest land deposits of comparable grade at Saldanha Bay. As the phosphatic fraction of the sediments south of Lüderitz is not likely to be $>16\%$ P_2O_5, the average for local source rocks[6], the southern deposits have no immediate economic interest.

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Tidal Resonance in the Coral Sea

THE "age of the tide" is a measure of the time lag between new or full Moon and the following spring tide. In the Coral Sea, the age of the tide is negative[1]. This behaviour is rare in the ocean. In terms of the tidal response function this means that there is a negative phase change in the $\bar{R}_2^2$ (ω) response function[2], between the frequencies of the dominant M_2 and S_2 constituents.

Suspecting that this may be the consequence of a resonance, I have tried fitting a response function of the form

$$\bar{R}_2^2\ (\omega) = A + B\omega + C/(\omega - D)$$

to the harmonic constants for Cairns[3]. The first two terms correspond to the background and the third term to the resonance. Only two terms were used for the background to reduce the number of free variables. I estimated the complex variables A to D by minimizing the expression

$$\sum_j (|h_j - \bar{R}_2^2(\omega_j)e_j|)^2$$

where h_j is the complex harmonic constant relative to Greenwich, for the semi-diurnal constituent with angular velocity ω_j and equilibrium amplitude e_j (ref. 4).

The function obtained in this manner is shown in Fig. 1. The data points are the values of the response functions at the twelve constituent frequencies calculated from the relation,

$$\bar{R}_2^2(\omega_j) = h_j/e_j$$

The error bars indicate the expected standard deviation due to noise in the original record[5]. For the constituents N_2, M_2, S_2 and K_2 the s.d. is less than the radius at the data points.

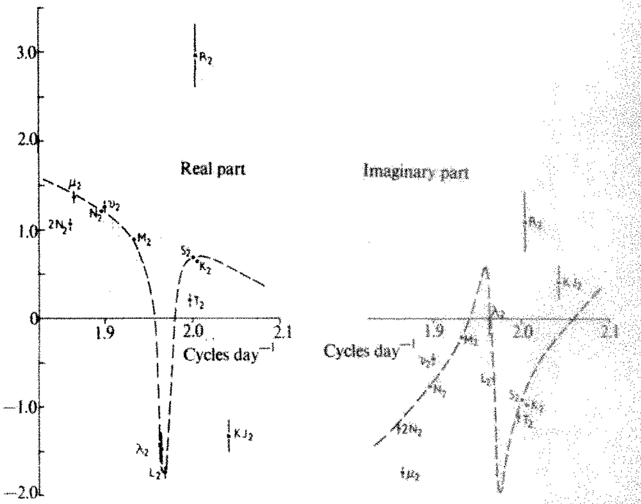

Fig. 1 Real and imaginary part of the R_2^2 (ω) tidal response function at Cairns (16° 55′ S, 145° 47′ E), showing the influence of a resonance in the Coral Sea.

The coordinate of the resonance is $1.969 - i\,0.009$ cycles day^{-1} with an expected s.d. in both coordinates of 0.003. The imaginary part corresponds to a decay time of 18 days.

Initially it also seemed possible that the large phase differences might be due to the radiational tide[2,6], but this would have meant that the radiational component of S_2 was much larger than its normal value of about 20% of the gravitational component. Further, the data of Fig. 1 show that the resonance hypothesis is supported by the behaviour of the L_2 and λ_2 constituents, which would not be affected by the radiational tide.

The existence of the resonance is also confirmed by calculations using harmonic constants for Cooktown[3] and Port Moresby[7]. But in these cases the fit to the harmonic constants is not as good as it is by Cairns. Non-linear interactions may, however, introduce an error of up to 25% in the L_2 constituent at Cairns.

If the resonance is an oceanic resonance and is not due to the movement of nearby land masses[8,9], then I believe this to be the first time an oceanic resonance has been observed lying within a tidal band. A fuller discussion of the method and of its errors will be published later.

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Origin of Bright Spots in High Resolution Dark Field Electron Microscopy

SMALL bright spots (0.3–0.7 nm) have been observed in the dark field images of evaporated carbon films[1] but their origin has not been explained. Here I suggest that these bright spots are present in the dark field image at an overfocus (a higher objective lens current) of 300 nm±50 nm relative to the bright field focus (the finest granulation in the phase structure).

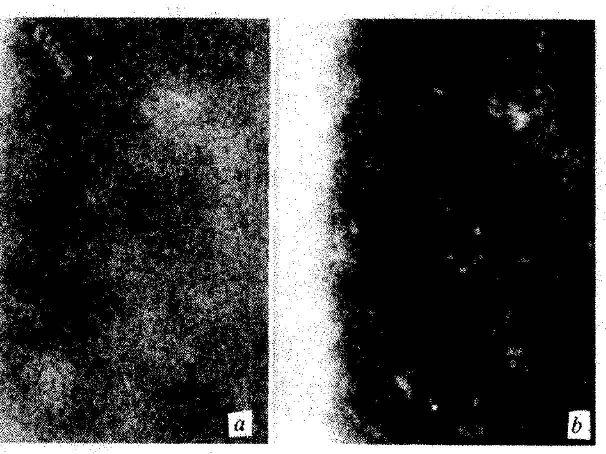

Fig. 1 *a*, Dark field image of a thin (3.5 nm±30%) carbon film taken at the objective lens current for bright field focus ($\Delta f=0$). *b*, Bright field spots at maximum sharpness at an overfocus of $\Delta f=\pm 300$ nm in respect to the bright field focus. Film magnification 95,000, final magnification 2.1×10^6.

According to Thon[2], there is then an image of a plane in the bright field which is slightly overfocused in relation to the geometrical or Gaussian image plane. For an electron microscope with a spherical aberration coefficient of 1.6 nm, such as the Philips EM300, this overfocus is 50 nm±30 nm. Bright spots therefore occur in the dark field image at an overfocus of 1.6 nm±80 nm in relation to the geometrical image plane. The dark field images of a carbon foil, obtained using conical dark field illumination[3], are shown at both focus conditions in Fig. 1. To contribute to the dark field image, the electrons were scattered through angles between α 0.0001 and 0.001 rad.

To account for the small size of the bright spots, so far unexplained, and for their observation at overfocus with respect to the bright field focus, a model is proposed based on a wave interference phenomenon well known from classical optics[4]. A plane wave passing a circular aperture of diameter *a* generates, at a point P behind the aperture, through constructive interference, a maximum with an intensity of four times the original intensity. The location of P is determined by the condition that the distance between P and the rim of the aperture exceeds the distance to the centre by half a wavelength. For the distance of P to the aperture (*b*), the relation $b=a^2\ (4\lambda)^{-1}$ can be calculated (λ=wavelength). From the location of the first dark ring in the interference pattern around P, one can show that the diameter at half intensity of the high intensity spot at P is somewhat less than one-third of the aperture diameter *a*. I think that a similar process produces the dark field bright spots, the wave fronts being generated by Bragg reflexion of electrons on small crystals present in the carbon film.

To substantiate this, the following points can be made.

First, inherent in the concept of Bragg reflexion is the idea that the outgoing wave from the crystal is a plane wave over the crystal area and therefore not very different from a plane wave passing an aperture.

Second, Ban *et al.*[5] have found indications that small graphite like crystals are present in carbon foils. Crystals with lattice spacings from 0.35 nm or greater (including graphite) can lead to Bragg reflexion inside the acceptance angle α of the dark field geometry. In general the "apertures" for the wave interference, formed by the crystals, will not be exactly circular and the high intensity maxima will occur at a distance corresponding to some average diameter of the crystals.

Third, the bright points are found at overfocus in relation to the geometrical focus. Relative to the direction of travel of the electrons, an image of a plane occurs behind the object, just where, according to the present model, one would expect the interference maxima.

Fourth, putting *b* equal to the defocus distance of 350 nm±80 nm, the dimensions of the crystals involved in the Bragg reflexion can be calculated from the formula $b=a^2\ (4\lambda)^{-1}$. For 80 keV electrons we have $\lambda=4.2$ pm (picometre) and, for the diameter *a* of the crystals, values in a range of 2–2.8 nm are found. One would expect the size of the high intensity interference maxima (the bright spots) to be somewhat less than one-third of this crystal size, about 0.5–0.7 nm. Smaller spots can be produced by photographic printing procedures. This was seen in photographs of a light optical analogue of the interference phenomenon. The observed size of the bright spots, 0.3–0.7 nm, is therefore in agreement with the spot size expected from the interference process, and thus constitutes an independent argument for this physical mechanism causing the dark field bright spots.

A full account containing bright and dark field through focus series will be published elsewhere, together with a consideration of the implications of the model for the dark field images on biological material in the electron microscope.

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Magnetic Fields, Ball Lightning and Campanology

WOODING suggests[1] that ball lightning is a plasma vortex ring structure produced by a process similar to the ablation of a solid surface by a high power laser pulse. A plasma vortex ring structure requires a magnetic field; here I present two pieces of evidence to show that a magnetic field is associated with ball lightning, and which may help to confirm this hypothesis.

Mühleisen[2] quotes a story of the disturbance of a ship's compass when a lightning ball was dancing about in the rigging; and the following story[3] would seem to indicate the presence of quite a powerful magnetic field.

"In the parish of Samford-Courtney in Devon on October 7, 1811, a sudden darkness came on, and a fire ball fell in the vicinity of the church. The ringers in the belfry, ringing at the time, declared that they never knew the bells go so heavy, and were obliged to desist ringing. Looking down from the belfry into the church, they perceived four fire balls, which suddenly burst, and the church was filled with fire and smoke, some of which ascended to the tower, where a large beam, on which one of the bells was hung, was broken, and the gudgeon breaking, the bell fell to the floor."

In England it is usual to ring the church bells in changes, each bell swinging through 360°, alternately clockwise and anticlockwise, at each pull of the rope; a speed of about twenty-five changes a minute is usual. The magnetic field strength necessary to stop the ringing of bells may be calculated as follows.

The potential generated when a disk of area A rotates at N revolutions s^{-1} in a magnetic field of strength H gauss is $NHA \times 10^{-8}$ V, $= 8.85 \times 10^{-5}\ H$ V, when $N = 0.5$ and $A =$ 17,700 cm^2, the radius from the line of gudgeons to the lip being taken as 75 cm for a bell of average size (15 cwt). The energy dissipated when an electric current flows in a conductor is V^2t/R W s. Setting $t = 2$ for one revolution of the bell (one pull of the rope).

Work done at each pull $= (8.85 \times 10^{-5})^2\ H^2\ 2/R = 100$ assuming that a ringer must stand when he must apply a force equivalent to more than 10 kg weight through 1 m or, say, 100 W s, at each pull to get the bell up to the balance. The generated potential will, I assume, produce eddy currents which flow against the resistance R. The resistance will be taken as that of the cross-section of the bell wall over a length of half the average circumference, $= 3.5 \times 10^{-6}\ \Omega$ when average diameter = 70 cm, average wall thickness = 7 cm, length of wall from lip to crown staple = 80 cm, and specific resistance of bronze = $18 \times 10^{-6}\ \Omega$ cm. For these numerical values, $H =$ 150 gauss.

This calculation can only be taken as an estimate because, first, the bell is not a rotating disk, but a compound pendulum consisting of a hollow vessel swinging through 360° although average angular velocity = 0.5 revolution s^{-1}, and, second, the resistance against which the eddy currents flow cannot be accurately calculated, as one does not know how the currents flow within the bell.

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HNCO in the Galactic Centre

The 1_{01}–0_{00} transition of the HNCO molecule at 21.9817 GHz has been detected using a 1.4 cm parametric amplifier at the 36 foot telescope of the US National Radio Astronomy Observatory[1]. The present observations were made with the same receiver and the 85-foot Maryland Point telescope of the US Naval Research Laboratory. The receiver has a single sideband system temperature of 600 K achieved by the use of a degenerate parametric amplifier with 20 dB of gain[2]. The filter bank receiver used to obtain the spectral line data consisted of 20 channels each 0.5 MHz in width giving a total velocity span of 135 km s^{-1}. At 1.4 cm the beamwidth of the telescope is approximately 2.1 arc min and the beam efficiency is 60%.

A map of the region around the galactic centre source Sgr B2 was made (Fig. 1). The spacing of the points was 2.0 arc min. The peak antenna temperature observed was 0.4 K and line velocity was 60 km s^{-1}. The shape of the contours is influenced by the sampling points which were taken east–west and north–south of the position of greatest line intensity.

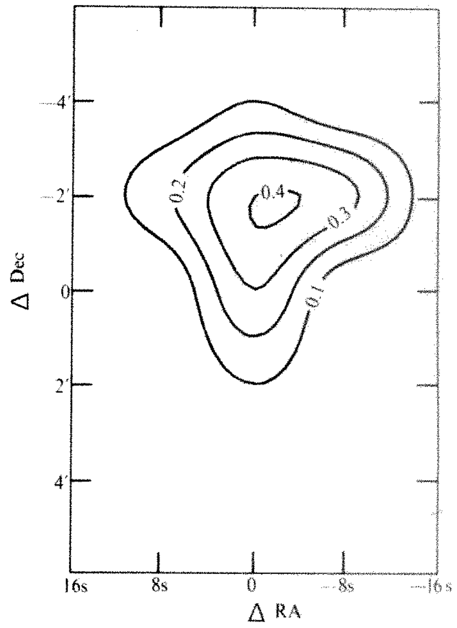

Fig. 1 Contour map of the distribution of HNCO in the galactic centre source Sgr B2. The offsets in RA and dec are from the central position RA (1950) = 17 h 44 min 11 s and dec (1950) = −28° 22′ 30″. The contour levels are antenna temperature.

The HNCO cloud appears to have a minimum diameter of ~5 arc min and is centred ~2 arc min north of the H_2O source Sgr B2(OH). A similar northern emission peak was found from mapping observations of both the 4_{04}–3_{03} line of HNCO at 87.9 GHz (ref. 3) and the 1_0–0_0 lines of CH_3OH at 48.3 GHz (ref. 4). The maximum intensity of the (3,3) line of NH_3 (ref. 5) and the highest optical depth of the 1_{11}–1_{10} line of H_2CO (ref. 6) are also observed in the direction of the HNCO emission peak. Thus this position should be searched for other molecular species to learn if it might be the principal region for molecular formation in Sgr B2 and hence of greater chemical interest than the well known Sgr B2(OH) position.

At a distance of ~10 kpc, the HNCO cloud has a diameter of ~15 pc. If we adopt 5×10^{14} cm^{-2} as the total projected density of HNCO (ref. 3), the total mass of HNCO is ~0.02 $M_\odot$. The molecular hydrogen density in the HNCO cloud is $N(H_2) \geq 1.8 \times 10^5$ cm^{-3} (ref. 3); thus the abundance of HNCO relative to H_2 is $N(HNCO)/N(H_2) \leq 6 \times 10^{-11}$ which agrees surprisingly well with $\sim 2 \times 10^{-11}$ which would be expected from the product of the cosmic abundances of the constituent atoms.

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Magnetoresistive Amplifier

THE construction of a bistable circuit with two magnetoresistors[1] raises expectations about other applications to active electronic circuits. The bistable circuit has some resemblance to valve and transistor circuits, but they are capable of acting as single element bistable multivibrators also; moreover, a single active element suffices for amplifiers and oscillators. Is the magnetoresistor equally versatile?

For a magnetic field B well below saturation, the resistance R is increased above an initial R_0 according to a square law

$$R = R_0 + aB^2 \tag{1}$$

If the current in the magnetoresistor is made to control its own magnetic field, rather than that of another, there is simply an increase in resistance, which is steep for an efficient magnetic circuit. A negative slope in the voltage/current characteristic is no longer possible at first sight.

But if a constant initial magnetic field is superimposed, the self magnetic field of the resistor can be directed against this initial bias. As the current now increases, resistance falls and the rate of fall can become so large that voltage/current curves slope negatively over part of their range.

$$R = R_0 + c(I_B - I)^2 \tag{2}$$

where the constant c includes a and the constants of the magnetic circuit; I_B is a bias current in a winding with the same number of turns as the winding which passes I through the magnetoresistor. If R_0 includes the resistance of that winding and V is the potential difference across the combination, then

$$V = IR_0 + cII_B{}^2 - 2cI^2 I_B + cI^3 \tag{3}$$

$$\mathrm{d}V/\mathrm{d}I = R_0 + cI_B{}^2 - 4cII_B + 3cI^2 \tag{4}$$

The condition for the occurrence of negative slope is found by equating (4) to zero and solving the quadratic; it is

$$R_0 \leq (c/3)I_B{}^2 \tag{5}$$

In Fig. 1A I show a magnetoresistor controlled jointly by a series winding and a separate bias coil. Figure 1B is a family of curves computed from equation (3) with bias field as parameter; Fig. 2 gives experimental negative resistance curves with the same equipment as the bistable circuit[1], except that four 500 Ω magnetoresistors are connected in series/parallel to act like a single 500 Ω element with a four-fold power rating. Depending on the choice of load line and external components, the element operates continuously at room temperature as an amplifier or an oscillator or a trigger circuit.

As an example of a d.c. amplifier, an operating point $V_{cd} = 20$ V, $I_c = 43$ mA is chosen with load at 210 Ω; in Fig. 1A terminals c and d are the output pair connected to a voltage source through the load resistance. Bias does not in fact require a separate winding, but is provided by passing a constant current (of 60 mA) from e to c. The winding ab is released to become the input pair of terminals. A power gain of 75 is recorded. The input may be imagined to create a small shift in the bias, which has a large effect on the operating point.

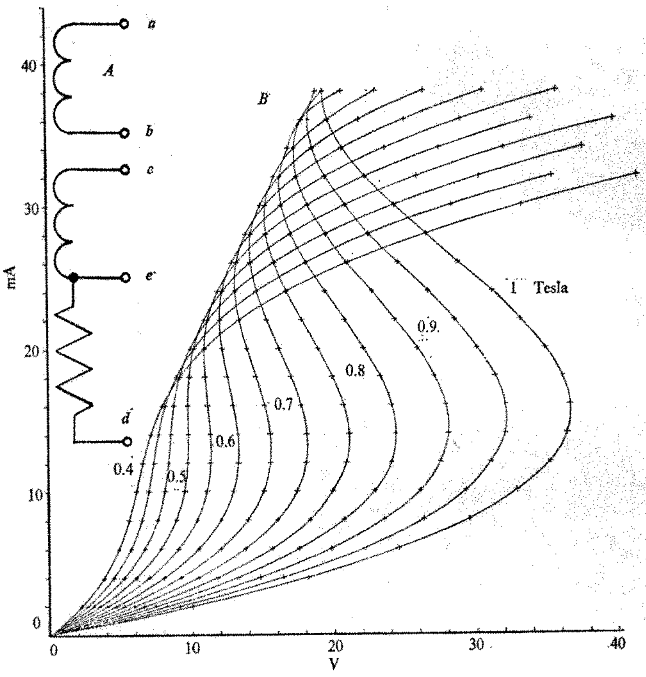

Fig. 1 A, Magnetoresistor with series control coil and separate bias coil. B, Current/voltage characteristics with bias field as parameter. (Computer graph plot. I_c against V_{cd}.

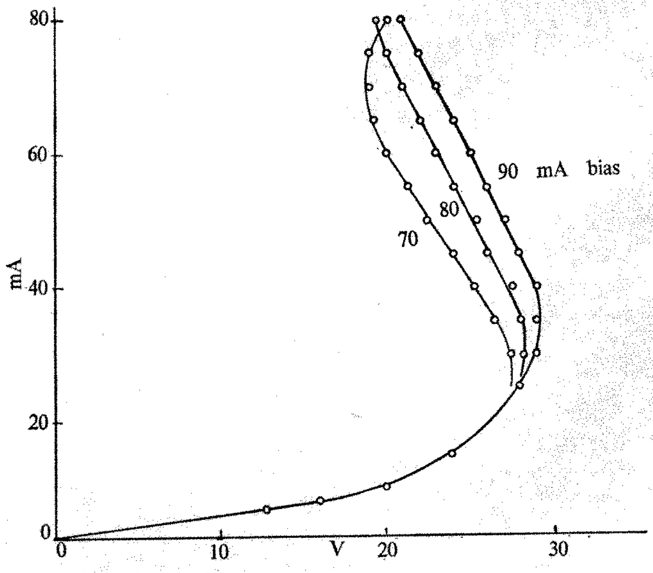

Fig. 2 Experimental current/voltage characteristics for a biased self-controlled magnetoresistor

An efficiency of 12% is measured, but if the bias had been provided by a permanent magnet 21% is indicated.

For an oscillator, a capacitor is connected between *c* and *d*, the bias is applied to *a* and *b* through chokes, and the power for the oscillator is supplied to *c* and *d* again through chokes, which have limited frequencies to 600 Hz so far. But if the magnetoresistor is a Corbino disk with no external circuit, natural frequencies of oscillation are observed at 400 MHz (ref. 2). The present theory is believed to apply to this example of self-excitation but there need be no series coil to augment the self-magnetic field of the disk[3].

The biased self-controlled magnetoresistor has interesting characteristics, which imply applications of wide versatility. The magnetoresistive effect is fundamental and does not wholly depend on the technology of high purity, single crystal semiconductors.

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BIOLOGICAL SCIENCES

DNA Differences between Flax Genotrophs

Certain environmental conditions can induce genetic changes in particular varieties of plants[1-5]; these changes are stable and inherited over many generations. One of the best examples of this involves the flax variety Stormont Cirrus, which when supplied with certain fertilizers and grown for at least 5 weeks in a heated greenhouse can change from its original form (P1) into a large stable genotroph (L), or a small stable genotroph (S), depending on the fertilizers supplied[1,6]. L plants may be up to six times the weight of S plants, depending on the environment in which they are grown, with the P1 type having a weight in between those of L and S.

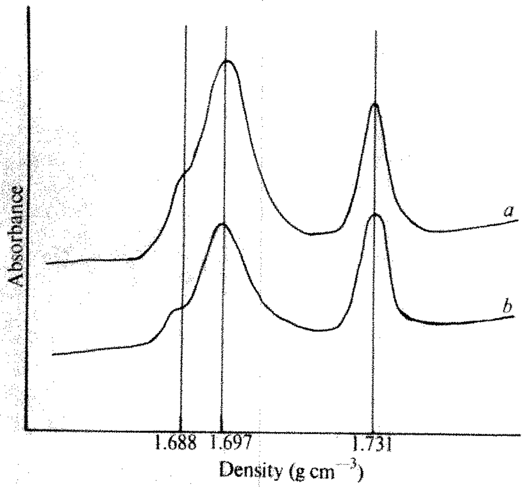

Fig. 1 Caesium chloride equilibrium gradient run for 24 h at 44,770 r.p.m. at 25° C on a Spinco Model E analytical centrifuge. *a*, 2 μg of DNA from L genotroph with a principal band of density 1.697 g cm^{-3} and a secondary band of density 1.688 g cm^{-3}. *b*, 1.5 μg of DNA from the S genotroph with a principal band of density 1.697 g cm^{-3} and a secondary band of density 1.688 g cm^{-3}. Both samples had 1 μg of DNA from *Streptomyces coelicolor*, density 1.731 g cm^{-3} added as a marker.

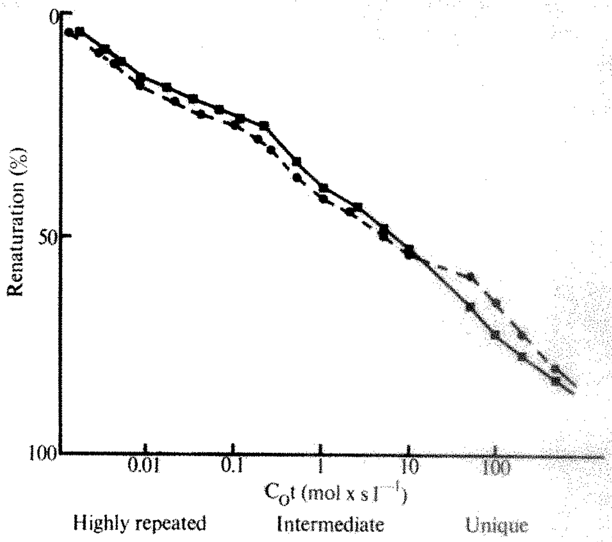

Fig. 2 C_0t curves of DNA from the L genotroph (■—■) and the S genotroph (● - - - ●). The DNA was sheared by sonication to a molecular weight of 7×10^5 and incubated at 50 μg ml^{-1} or 500 μg ml^{-1} in 0.12 M phosphate buffer (equimolar sodium dihydrogen phosphate and disodium hydrogen phosphate) at 60° C. Single and double stranded material was separated on a hydroxyapatite column also maintained at 60° C. The degree of renaturation was plotted against the C_0t value (ref. 8).

L and S behave as two distinct genetic types, giving equilinear inheritance when they are reciprocally crossed, and no transmission through reciprocal grafts[1]. L has been shown to have 16% more nuclear DNA than S as shown by Feulgen staining[6]. The P1 type has a DNA content intermediate between that of L and S, having 10% less DNA than L but 6% more DNA than S.

I have now characterized the nature of the DNA differences between the genotrophs, with the ultimate aim of attempting to elucidate the nature and mechanism of induction of the changes in DNA and in the phenotype. The method of extraction of DNA from the L and S genotrophs will be described elsewhere (my unpublished work). The extracted DNA was analysed by ultracentrifugation and renaturation. The DNA from both types formed two bands in neutral caesium chloride gradients (Fig. 1). The principal band had a density of 1.697 g cm^{-3} and a secondary band a density of 1.688 g cm^{-3}. The amount of DNA contained in the satellite band was approximately 15% and was about the same in samples from both types. The densities of both the satellite and the main band increased by 0.01 g cm^{-3} after heat denaturation.

The renaturation of the DNA was followed optically[7] and by separation on hydroxyapatite[8]. From the results (Fig. 2) it was apparent that the DNA from both L and S genotrophs contained some sequences which were highly repeated, others which were less repeated and some unique[8]. The highly repeated sequences make up 24% of the total DNA from L and 28.5% of the total in S (my unpublished work). The satellite DNA was included in this fraction. The difference between the proportion found in L and S could be explained as follows: if the extra DNA in L contained none of the highly repeated sequences, then the consequent dilution of such sequences in L would result in the differences observed.

The unique sequences form 45% of the total DNA in S and 40% of the total DNA in L; again this difference is accountable in terms of the dilution factor mentioned earlier. The complexity of the unique sequences, calculated against a value for *E. coli* of molecular weight 2.8×10^9 (ref. 9), is 2.1×10^{11}; this agrees well with the value calculated from the total DNA content per nucleus[10] which is $1.9^7\times^{11}$, assuming these unique sequences make up 45% of the total DNA in S and 40% of the total DNA in L.

The intermediate sequences, that is neither highly repeated nor unique, make up the remainder of the DNA which is 26.5% in S and 36% in L. This intermediate fraction in L seems to be composed of two families of sequences: one renaturing at about the same rate as the intermediate sequences in S; the other renaturing more slowly. The extra DNA in L could be in this latter fraction which was not observed in S.

An increase in the concentration of any type of sequence would cause an increase in the rate of renaturation of this sequence. The extra DNA in L was found in the slowest renaturing portion of the intermediate sequences and only the unique sequences renature more slowly than this. Thus the extra sequences must have been derived by increasing some of the unique material. In that case the difference in the rates of renaturation between the additional intermediate and unique material suggests that the extra DNA is present at seventy times the multiplicity of the unique sequences. This means that the extra DNA in L would be equivalent to 0.4% of the unique sequences at a multiplicity of seventy times.

How this increase in DNA is brought about by the fertilizer treatments is still unknown. But specific portions of the genome have been shown to be differentially increased or decreased, and the difference inherited, in other systems; for example in *Drosophila* the number of ribosomal genes at a particular locus can be increased or decreased depending on the chromosomal constitution[11]. Although this effect is not caused by an external treatment, a mechanism must exist to preferentially increase and stabilize a portion of the genome and in flax something similar may occur with parts of the genome.

I thank Dr A. Durrant for P1, L and S genotrophs.

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Environmentally-induced Changes in the Brains of Elderly Rats

ANIMALS that have been exposed to environmental stimulation develop marked differences in brain structure and biochemistry when compared with animals that have not been exposed in this way[1–4]. Factors influencing the magnitude of these changes are the duration of rearing and the age of the animals when the environmental separation takes place. Most studies have focused on young animals because the brain is thought to be more plastic at this stage. As a result, temporal analyses of the induced changes have usually been limited to fairly short periods of environmental exposure early in the animal's life. Rosenzweig *et al.*[3] have suggested that, in rats differentially reared from weaning, the induced differences in brain weight reach a maximum during the first 30 d of separation, after which they decrease. But the evidence for this biphasic response has been based on relatively short periods of differential rearing (15–160 d).

The aims of our study were two-fold. First, to test whether the early cerebral changes induced in weanling rats could still be detected after longer periods of environmental separation; second, to determine whether the brain remains capable of morphological change with increasing age. Rosenzweig *et al.*[5] found that the brains of 105-d-old rats were as easily modified as those of younger weanling animals, whereas Riege[6], using a much longer period before environmental separation (300 d), found evidence of a decreased rate of brain change in response to the differential rearing conditions. This latter study suggested that the potential for morphological change may indeed diminish in older animals.

Twenty litter-mate pairs of Wistar albino rats were matched for body weight and divided into two groups at weaning. The first group was divided equally between two open-mesh cages, both of which were supplied with an assortment of "toys" which were changed daily (enriched condition). Rats from the second group were placed singly into small stainless steel boxes with solid sides (isolated condition). Both groups were kept in their respective environments for 509 d, after which they were sub-divided equally into those animals that were to remain unchanged in their respective environments (enriched and isolated) and those that were to be tested on a Hebb-Williams maze (enriched, tested and isolated, tested).

The behavioural testing period took 21 d, during which time the tested animals were removed from their respective environments for 15–30 min once each day and then returned. During maze testing animals were deprived of water for 23 h for reward purposes. Both non-tested groups were maintained on precisely the same schedule. This short daily interval of behavioural testing and attendant handling therefore constituted the only difference between the tested and non-tested groups.

The life expectancy of these Wistar rats is uncertain but it is probably less than 900 d, so that by the end of the experiment the animals had lived for well over half of their expected life span. At the end of the testing period all rats were weighed and killed and their brains were removed, weighed and stored in 10% formalin.

Whole brain weight was obtained by removing the olfactory lobes anteriorly, and posteriorly by a vertical cut immediately behind the cerebellum. The brain was then sectioned coronally immediately behind the posterior pole of the cerebrum, so providing an anterior component which included all of the forebrain and most of the midbrain (forebrain sample) and a posterior rhombencephalic component which consisted mainly of cerebellum and medulla (hindbrain sample). These samples were weighed. After fixation for 1 month in formalin solution the length (from anterior to posterior pole) and the greatest width of the cerebrum were measured in the forebrain sample using a vernier scale at a magnification of ×12.5. All measurements were performed blind.

The results of the maze testing showed that enriched, tested animals made significantly fewer errors than isolated, tested ones, indicating that the difference in learning ability had been maintained throughout the protracted rearing period. Cerebral changes were analysed by an analysis of variance (ANOVA), with environment and testing as the two primary fixed factors.

Both environment and behavioural testing exerted a significant effect on brain weight (Table 1). The whole brain weights of the enriched animals were significantly heavier than those of the isolated (3.09%; $P<0.0125$) and this difference was entirely due to the forebrain sample. The hindbrain sample showed a non-significant trend in the reverse direction, thereby making

Table 1 Mean Brain Weights (g) after Differential Rearing for 509 Days and Behavioural Testing for 21 Days

	Whole brain	Forebrain	Hindbrain	Forebrain hindbrain
Isolated	1.778	1.276	0.502	2.552
Enriched	1.860	1.365	0.494	2.767
Isolated, tested	1.855	1.328	0.526	2.534
Enriched, tested	1.886	1.377	0.508	2.714
Isolated against enriched				
% diff.	3.09	5.30	−2.55	7.75
$P<$	0.01	0.002	N.S.	0.002
Non-tested against tested				
% diff.	2.81	2.46	3.75	−1.35
$P<$	0.05	N.S.	N.S.	N.S.

the forebrain : hindbrain ratio a sensitive index for the detection of environmentally induced change. This agrees with previous findings[4].

Testing also increased whole brain weight (2.81%; $P<0.05$) but the increase was not as clearly localized in the forebrain sample. None of the four possible testing × environment interactions were significant for brain weight.

Since brain weight depends partly on body weight[3], the effect of the latter was removed by analysis of covariance. This did not significantly alter the primary effects indicated by the ANOVA in Table 1, but it did alter the testing × environment interactions, making them significant in the case of the whole brain ($P<0.01$) and forebrain ($P<0.001$). The interactions for hindbrain and for forebrain/hindbrain remained non-significant. Both significant interactions were in the same direction as that shown for forebrain (Fig. 1), indicating that the effect of testing in increasing either whole brain or forebrain weight was apparent only in the previously isolated animals.

Cerebral dimensions also showed significant changes (Table 2). The cerebral cortices of the enriched subjects were 1.38% longer ($P<0.025$) than those of their isolated counterparts, and while neither the width measurements nor any of the changes due to behavioural testing were significant, there was a significant testing × environment interaction ($P<0.05$) for the derived parameter of cortical length × cortical width (Fig. 2).

These results agree substantially with previous studies[4,7] in which cerebral length, but not width, was significantly modified by differential rearing. But the combination of length × width represented the most sensitive measure of changes in cerebral dimension.

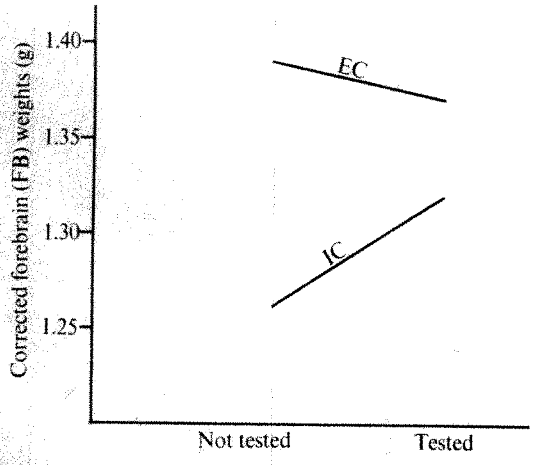

Fig. 1 Testing × environment interaction on the forebrain weight after a covariate adjustment for body weight. The effect of testing on the enriched animals had no significant effect on forebrain weight (enriched > enriched, tested by 1.25%; N.S.) but testing had a marked effect on the forebrain weight of the previously isolated animals (isolated, tested > isolated by 5.16%; $P<0.005$). The interaction for the whole brain sample was very similar.

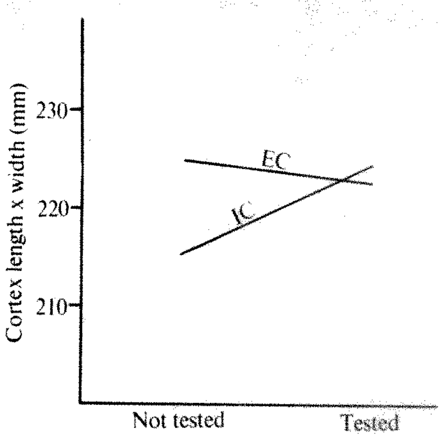

Fig. 2 Testing × environment interaction on the derived parameter of cerebral length × cerebral width. Testing had no significant effect on the enriched animals (enriched > enriched, tested by 0.87%; N.S.) but testing had a marked effect on the length × width of the previously isolated subjects (isolated, tested > isolated by 4.65%; $P<0.05$).

Analysis of covariance, using body weight as the covariate, did not significantly alter the results obtained by the original ANOVA, but the use of the forebrain weight as the covariate significantly altered the original difference so that enrichment no longer influenced either cerebral length or cerebral length × width. But the test × environment interaction on the product of length × width remained significant as before (enriched > enriched tested by 1.04%; N.S., and isolated, tested > isolated by 3.89%; $P<0.05$).

Table 2 Mean Cortical Dimensions (mm) after Differential Rearing for 509 Days and Behavioural Testing for 21 Days

	Cortex length	Cortex width	Length × width
Isolated	14.46	14.87	214.64
Enriched	14.87	15.19	225.85
Isolated, tested	14.73	15.25	224.63
Enriched, tested	14.73	15.19	223.87
Isolated against enriched			
% diff.	1.38	0.87	2.37
$P<$	0.025	N.S.	0.0125
Non-tested against tested			
% diff.	0.43	1.22	1.81
$P<$	N.S.	N.S.	N.S.

Two principal conclusions emerge. First, that many of the environmentally induced structural and behavioural changes induced in the developing animal can be maintained for a very much longer period than was previously thought probable, and second, that the rat's brain retains its capacity for structural change into old age. On the latter point it would seem that the long period of stimulus deprivation may have actually made the isolated animals more susceptible to stimulation since similar periods of behavioural testing have not been shown to induce changes of such magnitude in young animals[3]. Certainly the isolated animals showed a marked aversion to handling during the first few days of behavioural testing and generally seemed to be more aroused during each test period than their previously enriched littermates.

Evidence for structural plasticity was supported by changes in both brain weight and cerebral dimensions in response to sensory stimulation from either the rearing environment or the testing situation. It is of interest, however, that there did seem to be some ceiling effect imposed upon the environmentally-induced changes since the period of testing had no detectable effect on the previously enriched brains and only succeeded in increasing the size of the previously isolated brains to that of the enriched. Such a ceiling effect is consistent with the hypothesis that these neurological changes were the result of a genetic ×

environment interaction such that the extent of the neurological adaptation to a stimulus-rich environment was limited by a genetically imposed maximum; a maximum which had, presumably, been reached by the enrichment phase alone.

The resulting picture is, therefore, one of a brain dynamically reacting to an increased level of environmental stimulation by neurological change, indicating that the rat brain retains a considerable degree of structural plasticity into old age.

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Acetylcholine Release within the Cat Striatum during the Sleep-Wakefulness Cycle

THE caudate nucleus (CN) is a subcortical region rich in acetylcholine (ACh)[1]. Considering the role played by this nucleus[2] in sensorimotor control, changes in its cholinergic activity could occur during different stages of sleep (S) and wakefulness (W). Involvement of the CN in the S-W cycle is likely as electrical stimulation of the neostriatum results in S in various species[3-5]. We have therefore investigated the ACh output from caudate neurones during W, slow-wave sleep (SWS) and paradoxical sleep (PS) using chronic push-pull cannulae.

In five cats of either sex (2.5–3.0 kg), the following electrodes were implanted under pentobarbital anaesthesia (35 mg kg^{-1} intraperitoneally): (1) Bipolar electrodes (0.4 mm diameter) in one lateral geniculate body (coordinates: $L=10$, $A=6$, $H=+2.5$ (ref.6)); (2) bilateral cortical (occipital) screw electrodes; (3) bilateral EMG electrodes in the lateral rectus muscle of both eyes; (4) bilateral EMG loop electrodes in the neck muscles. A concentric push-pull cannula (internal diameter of the outer and inner cannula = 0.9 and 0.2 mm respectively) was inserted in the head of one CN ($A=16$, $L=3.8$, $H=+4.5$ (ref. 6)).

Three to six days later recording and perfusion sessions were performed with the animal in a ventilated, sound-attenuated chamber ($60\times60\times60$ cm). EEG and EMG were recorded by an eight-channel Grass polygraph. The CN was perfused through the push-pull cannula at 0.1 ml min^{-1} with a warmed physiological Ringer-solution by means of a constant pressure perfusion pump (Ismatec M13). Individual 2 min samples were collected in a chilled fraction collector and ACh was determined radioenzymatically as described previously[7]. In some preliminary experiments neostigmine added to the perfusion fluid (5×10^{-4} M) did not result in changes in ACh output and so was not further used. After one or two perfusion sessions each animal was killed by an overdose of pentobarbital and the localization of the cannula and electrodes verified histologically. Output of ACh was correlated with the concomitant changes in behaviour, EEG and EMG, characterizing W, SWS and PS according to the commonly accepted criteria[8,9].

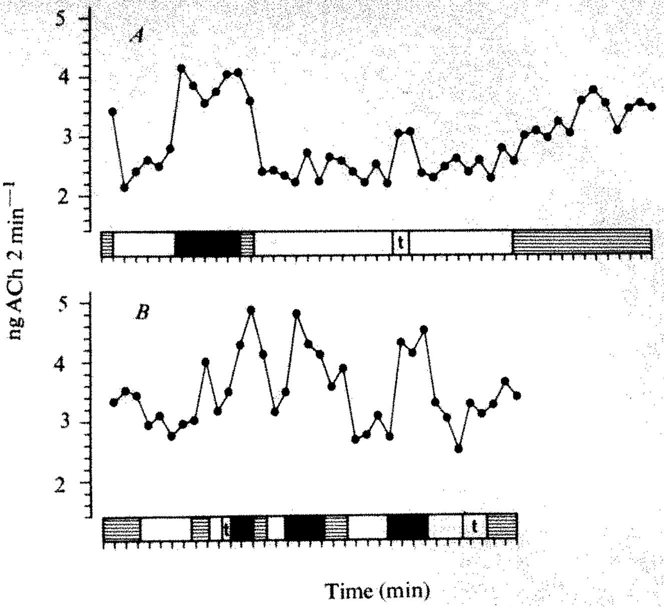

Fig. 1 Output of acetylcholine from the caudate nucleus as related to the sleep–wakefulness cycle during two different (*A* and *B*) perfusion sessions in one cat. The intervals on the abscissa indicate 2 min. (Transition = polygraph pattern intermediate between SWS and PS (*A*) or phase changes during a single sampling (*B*).) (▤), wakefulness; (□) slow-wave sleep; (■), paradoxical sleep; ([t]), transition.

The output of ACh from the CN and the phases of the S-W cycle during two typical perfusion experiments in one cat are shown in Fig. 1. Figure 1*A* depicts relatively prolonged, homogeneous phases of W, SWS and PS, whereas Fig. 1*B* illustrates shorter and more rapidly fluctuating phases. In all instances, ACh output decreased during SWS and increased during PS when compared to W. These changes occurred rapidly and in parallel to those of the electrical activities, and lasted as long as the various phases, irrespective of their duration. The statistical results are presented in Table 1.

It is evident that the differences in ACh output during W, SWS and PS were not only qualitatively the same in all cats,

Table 1 Output of ACh from the Caudate Nucleus of Cats during Wakefulness, Slow-wave Sleep and Paradoxical Sleep

Cat	Perfusion sessions	W ng/2 min	SWS* ng/2 min	PS* ng/2 min
1	*A*	3.12±0.08 (32)†	2.10±0.10 (19)	—
2	*A*	2.60±0.12 (14)	2.24±0.12 (12)	3.88±0.12 (14)
	B	3.38±0.08 (22)	2.32±0.10 (12)	4.08±0.12 (26)
3	*A*	3.80±0.06 (5)	—	4.50±0.06 (6)
	B	3.92±0.14 (18)	3.20 (3.12, 3.28)	5.92±0.18 (8)
4	*A*	3.34±0.06 (12)	2.44±0.08 (22)	3.90±0.08 (6)
	B	3.58±0.08 (9)	2.84±0.06 (17)	4.58±0.10 (8)
5	*A*	3.18 (3.22, 3.14)	2.54±0.08 (8)	4.42±0.06 (7)
Mean of the light perfusion sessions		3.38±0.16	2.52±0.16	4.46±0.30

* All values during SWS and SP are significantly different from those during W ($P<0.01$) either within each experiment (in which five or more samples were obtained) or as mean of the eight perfusions.

† Number of determinations indicated in parenthesis. For sessions in which less than five determinations were available individual values are given.

but also, and most importantly, the magnitude of these changes and the absolute ACh output during each phase were quantitatively similar. Thus, the output of ACh from the CN is greatly dependent upon the state of the W-SWS-PS cycle. The 2 min sampling period was too long, however, to measure accurately the biochemical changes during rapid transition between phases. In fact, when these periods occurred in the middle of a single sample collection, the corresponding ACh values were intermediate between those of the preceding and the following phases (Fig. 1*B*). This indicates further that the ACh output varies rapidly during changes in the W-SWS-PS cycle. Another type of transition occurred when a change from SWS to PS failed to develop fully, resulting in ACh release which was also intermediate between those of SWS and PS (Fig. 1*A*).

Previously, the ACh released from the cortex into a perfusion cup had been determined only in the presence of ACh-esterase inhibitors[10,11]. In our experiments, these compounds did not influence the release of ACh from the CN, possibly due to a rate of wash-out of ACh from the tissue greater than its hydrolysis.

The involvement of ACh in the S-W cycle is indirectly suggested by previous reports. Thus, during SWS the release of ACh from the cerebral cortex decreases as compared with that during W and PS[10,11]. Application of ACh crystals in brain regions, including the striatum[12], induced behavioural and electrographic manifestations of S. Moreover, the neostigmine-induced cortical desynchronization accompanying states of W was blocked by atropine[11,13] which was also able to prevent the appearance of PS[8]. Microinjections of ACh into the brain stem reticular formation caused EEG synchronization and S[14]. The participation of the CN in the W-SWS-PS cycle has some basis from previous studies. This structure may counteract the action of the ascending reticular formation and thus intervene in the mechanism of W[15]. Both electrical stimulation[3-5] of, and ACh application[12] into, the CN in different species induces a state of motor inactivity and eventually S.

This is the first study to examine the release of ACh from a subcortical structure (CN) during physiological changes in the chronically implanted, unrestrained, non-medicated cat. We have related the concepts of cholinergic and striatal involvement in the W-SWS-PS cycle and shown directly that the ACh output within the CN is smaller in SWS and greater in PS than in W. In addition, the rate of release of ACh during a phase of homogenous electrical activity (W, SWS or PS) is remarkably constant. These results may explain some clinical findings: thus, in extra-pyramidal disorders involving an imbalance between cholinergic and monoaminergic neurones (for example, Parkinson's syndrome)[16,17] many symptoms remit during S[18,19]. This may be the result of reduction of ACh release during SWS.

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Development of Glucose Oxidation in Isolated Nerve Endings

A HIGH proportion of oxidative metabolism in the cerebral cortex is thought to occur in dendritic areas which are rich in synapses[1,2]. With the development of methods to prepare isolated nerve endings (synaptosomes) from brain[3], it has become possible to investigate the characteristics of oxidative metabolism at the synapse without significant glial contamination[4,5]. Glucose is taken up into synaptosomes by a high-affinity carrier-mediated transport process[6] and subsequently oxidized to support synthesis of ATP and phosphocreatine[4,5]. Mitochondria probably do not metabolize glucose directly[4,5] and glucose transport and metabolism are largely abolished if synaptosomes are ruptured by hyposmotic shock[4,6].

Isolated nerve endings can be used to study the effect of added cation on transport and metabolism because, unlike brain slices, synaptosomes are relatively stable and metabolically active in buffered sucrose. We have learned that Na^+ added to the incubation medium is not required for transport of 2-deoxy-D-glucose into synaptosomes[6]. By extrapolation from this observation, the findings reported here suggest that added Na^+ stimulates glucose oxidation by 180% after glucose has entered nerve endings prepared from adult brain, and the primary effect is not on transport itself. Also, Na^+ stimulation of glucose oxidation is minimal in synaptosomes prepared from immature brain but is acquired during maturation by respiring synaptosomes.

Synaptosomes were prepared from rat brain[3,7] and inspected by electron microscopy by Dr J. R. Baringer of this department. They resembled synaptosomal fractions in published reports. Aliquots of synaptosomal suspension (0.4 ml) containing 2 mg protein were incubated in duplicate in 2.1 ml of a solution,

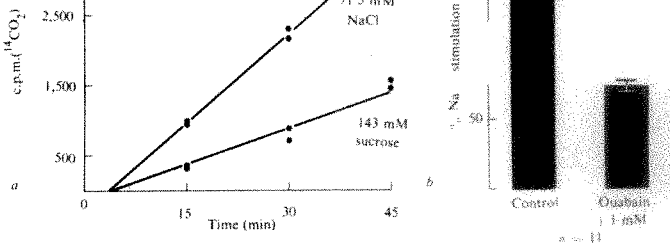

Fig. 1 *a*, Time course for glucose oxidation in rat brain synaptosomes in the presence or absence of Na^+. Conditions are as described. *b*, Effect of ouabain on the percentage Na^+ stimulation of glucose oxidation. The percentage is calculated by subtracting 1 from the quotient c.p.m. (71.5 mM Na^+) ÷ c.p.m. (143 mM sucrose) and multiplying by 100.

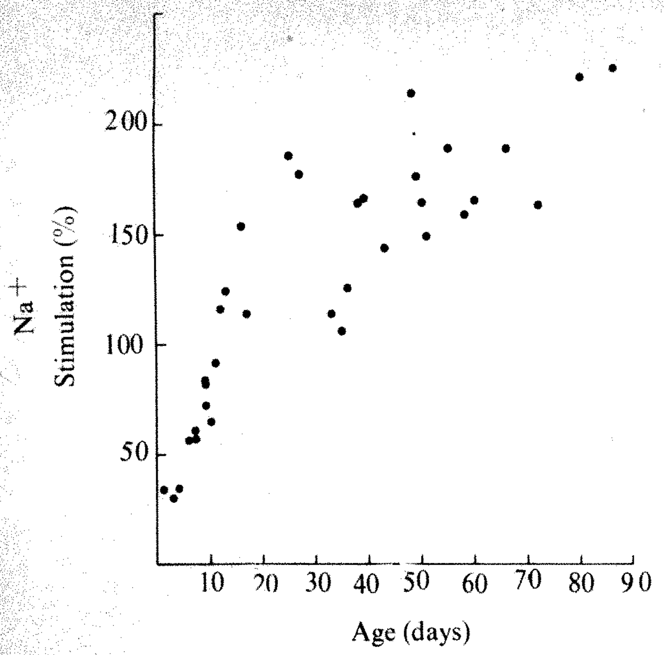

Fig. 2 Development of glucose oxidation in rat brain synaptosomes. The percentage Na^+ stimulation is calculated as in Fig. 1. Each point represents the average of duplicate measurements from a single animal or brain pooled from the same litter.

*p*H 7.4, containing 71.5 mM NaCl, 47.6 mM sucrose, 24.8 mM potassium phosphate buffer and 9.5 mM U-^{14}C-glucose, 3.8×10^7 c.p.m. mmol^{-1}. Control flasks did not contain synaptosomes. Incubation was carried out in stoppered 25 ml Erlenmeyer flasks (Metalglass Co., Boston) in a rotary water bath at 150 r.p.m. for 30 min at 37° C. Flasks had a centre well which contained a pleated 3×2 cm Whatman No. 1 filter paper saturated with 0.1 ml of 10% KOH to trap evolved $^{14}CO_2$. The reaction was stopped by injecting 0.5 ml of 0.5 M H_2SO_4 through the serum stopper into the incubation mix surrounding the centre well. After equilibration for 30 min filter papers were transferred directly to counting vials containing a 'Triton'-toluene scintillation solution for determination of radioactive $^{14}CO_2$ retained on the paper. Recovery of evolved $^{14}CO_2$ from ^{14}C-carbonate was nearly 100% and the assay was linear for protein concentration.

When synaptosomes from adult rats were incubated with ^{14}C-glucose, evolution of $^{14}CO_2$ was linear to 40 min whether or not NaCl (71.5 mM) was substituted for sucrose (143 mM) (Fig. 1*a*). Addition of $MgCl_2$ (1 mM) was without effect. Addition of NaCl resulted in a 180% stimulation of glucose oxidation (Fig. 1*b*). This effect appeared to be specific, for Na^+ as KCl and choline Cl at the same concentration were without effect.

Glucose oxidation may be increased in the presence of Na^+ to replenish ATP consumed by Na^+-K^+ATPase. As ouabain inhibits Na^+-K^+ATPase, ouabain should inhibit Na^+-dependent glucose consumption in synaptosomes without affecting glucose oxidation in the absence of Na^+. Ouabain (1 mM) did not alter glucose oxidation by synaptosomes in the absence of Na^+ in eleven separate experiments. In the presence of Na^+, however, ouabain reduced Na^+-stimulated glucose oxidation by 60% (Fig. 1*b*). These results suggest that Na^+-dependent glucose oxidation in synaptosomes may be linked to membrane Na^+-K^+ATPase and ADP production. 2,4-Dinitrophenol (0.1 mM) stimulates glucose oxidation in synaptosomes by 379% when regulation by ADP is removed. Thus, nerve endings probably have respiratory control, and fluctuations in Na^+ which attend changes in excitation and synaptic transmission may play an important role in regulating energy metabolism in nerve endings. Similar conclusions have been reported by Verity using glutamate[5].

Nerve endings contain Na^+-K^+ATPase, and there is an increase in synaptosomal Na^+-K^+ATPase activity with maturation[5,8]. We explored the development of Na^+-dependent glucose consumption in nerve endings by preparing synaptosomes from the brain of developing rats. There was a rapid increase in Na^+-dependent glucose oxidation with development, but glucose oxidation in the absence of Na^+ did not change as dramatically with age. These results are expressed as the percentage of glucose oxidation stimulated by Na^+ (71.5 mM) compared with sucrose (143 mM) (Fig. 2). The marked increase in this percentage with maturation cannot be explained by recovery of greater numbers of synaptosomes with age. Development of Na^+-dependent glucose oxidation correlates roughly with the appearance of Na^+-K^+ATPase in the synaptosomal fraction[8]. Several possibilities may account for the development of Na^+-dependent glucose oxidation in isolated nerve endings. During maturation different populations of synaptosomes might be recovered from the brain at different stages of development, there may be activation of a latent form of Na^+-K^+ATPase in nerve endings, or during development newly synthesized Na^+-K^+ATPase may be inserted separately into existing synaptosomal membranes.

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Effect of Lithium on Brain Dopamine

THE efficacy of lithium ion in the therapy of mania is now established[1,2], and much speculation about its mode of action has involved its effects on brain catecholamine metabolism. Treatment with lithium ion increases the turnover rate of whole brain noradrenaline[2-4]; increases intraneuronal while decreasing extraneuronal metabolism of noradrenaline[3,4]; increases uptake of noradrenaline by synaptosomes[5], and reduces the rate of release of ^{3}H-noradrenaline caused by electrical stimulation of striatal slices[6]. The influence of lithium ion on brain dopamine, however, is less fully documented. Here we describe the effect of chronic lithium chloride treatment on dopamine synthesis in striatal brain slices.

Male albino Sprague-Dawley rats (180 to 200 g) were housed four per cage in a regime of 12 h light and 12 h darkness and fed lab chow and water *ad lib.* Lithium chloride, dissolved in distilled water (0.7% w/v) or an equal volume of isotonic sodium chloride, was administered intraperitoneally (i.p.) as acute or chronic daily injections. Animals were killed 60 min after the last injection, the brains were quickly removed, rinsed in ice-cold Krebs-Henseleit physiological solution and the striatum was dissected out as described by Glowinski and Iversen[7]. Striatal slices (0.4 mm thick) were prepared using the slicing guide described by McIlwain[8] and placed in flasks containing 2 ml. of oxygenated cold physiological solution. Incubations were carried out at 37° C in an atmosphere of 95% O_2–5% CO_2. After a 10 min preincubation, 3,5-^{3}H-tyrosine (New England Nuclear Corp.) was added to each flask to give a final concentration of 8.15×10^{-6} M tyrosine and the incubations were continued for a further 45 min. The tissue and incubation media were rapidly separated by filtration and the tissue slices were washed twice with cold physiological solution. The tissue was homogenized in 0.4 N perchloric acid and the media and washings were acidified to give a final 0.4 normality with perchloric acid. The samples were centrifuged and cold tyrosine and dopamine were added to the supernatant as carriers. Labelled and endogenous tyrosine and dopamine were isolated on 'Dowex' 50-4X columns (K^+ form) followed by purification with alumina as described by Neff *et al.*[9]. Aliquots of the tyrosine and dopamine were taken for radioactive determination by liquid scintillation spectroscopy. Endogenous amines were determined fluorometrically by methods described previously[10,11]. Fluorescence was read in the Aminco Bowman spectrofluorometer. Plasma lithium concentrations were determined by atomic absorption spectrometry.

Table 1 Effect of LiCl on Dopamine Synthesis in Striatal Slices

Treatment	N*	Dose (mequiv kg^{-1})	Plasma Li$^+$ (mequiv l.$^{-1}$)	Dopamine (µg g^{-1})	^{3}H-Dopamine (c.p.m. mg^{-1})
Acute NaCl	16	—	—	4.21±0.08	1,063±21
LiCl	10	2	1.65	4.34±0.05	1,108±28
LiCl	10	4	2.84	4.28±0.06	1,162±31
Chronic NaCl	8			4.02±0.07	1,160±44
LiCl	8	1	0.64	3.89±0.07	761±25†
LiCl	8	2	1.52	3.82±0.05	445±24‡

*Number of animals; each provided duplicate striatal samples.
†$P < 0.005$.
‡$P < 0.001$.

Acute injections of lithium chloride in doses of 2 to 4 mequiv kg^{-1} did not affect endogenous dopamine nor the synthesis of ^{3}H-dopamine in striatal slices (Table 1). Daily administration of lithium chloride, 1 and 2 mequiv kg^{-1} for 14 days, inhibited striatal dopamine synthesis by 34 and 62% respectively (Table 1). These doses caused a slight but insignificant decrease in endogenous dopamine. There was no alteration in tyrosine specific activity. Serum lithium concentrations of 0.54 to 1.7 mequiv l.$^{-1}$ resulted from the chronic treatment; these levels are within the range associated with lithium therapy. The *in vitro* addition of 4 to 18 mequiv l.$^{-1}$ of lithium ion, or the replacement of similar amounts of sodium by lithium in the media incubating striatal slices obtained from saline-treated rats, did not affect dopamine synthesis (Table 2).

Table 2 Effect of *in vitro* LiCl or Replacement of Na by Li Ion on Striatal Dopamine Synthesis

Concentration of Li$^+$ (mequiv l.$^{-1}$)	N*	^{3}H-Dopamine (c.p.m. mg^{-1})
Added to control media	6	948±24
4	4	1,028±34
6	4	1,006±29
Replacement of Na	6	
4	4	959±28
6	4	1,078±31
18	4	992±32

*Number of animals; each provided duplicate striatal samples.

Thus the inhibition of dopamine synthesis by lithium ion seems to require chronic treatment. High lithium concentrations produced by either an acute injection or by *in vitro* addition of lithium ion are insufficient to inhibit synthesis. These conclusions correlate with the clinical observations that lithium's anti-manic action requires 1 to 2 weeks of daily medication. Our results agree with the observed decrease of dopamine excretion in manic patients undergoing lithium treatment[12]. Although the clinical results may reflect the effect of lithium on the peripheral disposition of dopamine, the data reported here suggest that central dopamine metabolism is altered in the same direction by lithium treatment.

Corrodi *et al.*[14] also found a slight but significant decrease in whole brain dopamine turnover in rats treated with lithium for 3 weeks, judged on the basis of the rate of dopamine decline observed after injection of α-methyl-*p*-tyrosine. They have further found, using the histochemical fluorescence method, an increase in tubero-infundibular dopamine turnover. On the other hand, Ho *et al.*[15] found no alteration in dopamine turnover rates in four brain regions after 4 weeks of treatment with lithium. They used the synthesis inhibition method for estimating dopamine turnover. Although differences in methods and treatment time may have contributed to the discrepancy in these results, the fact that different brain regions were studied may be more significant, especially as we have been concerned only with the corpus striatum.

Messiha *et al.* reported increased urinary levels of dopamine during manic states[12], while others have described the induction of hypomanic and manic behaviour by L-dopa in depressed patients with prior episodes of mania[13]. Thus, the inhibition of striatal dopamine synthesis may play some part in the psychomotor action of lithium in mania, or this element may exert some more fundamental action on the disorder itself.

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L-Glutamic Acid Decarboxylase in Parkinson's Disease: Effect of L-Dopa Therapy

IN Parkinson's disease severe decreases occur in striatal L-dopa decarboxylase, putaminal synaptosomal uptake of dopamine, and dopamine and homovanillic acid concentrations in the nigro-striato-pallidal complex[1–4]. In addition, L-glutamic decarboxylase (GAD) activity is decreased in the caudate nucleus[5]. The experiments described here were performed to further evaluate this finding and to determine the effect (if any) of L-dopa therapy.

Table 1 Activity of L-Glutamic Acid Decarboxylase in Discrete Brain Regions of Control Patients and Patients with Parkinson's Disease

Caudate nucleus	Putamen	Pallidum externum	Substantia nigra	Temporal cortex
A, Control patients				
1,318±185 (13)	1,243±220 (13)	1,106±306 (13)	1,273±297 (13)	1,024±154 (13)
B, Patients with Parkinson's disease				
1. No L-dopa therapy				
641 (2)	583 (2)	776 (2)	526 (2)	663 (2)
2. L-Dopa therapy for 8 months or less (average dose: 3 g day^{-1})				
339±53 (4) *	249±24 (4) *	504±50 (4)	300±102 (4) †	292±101 (3) *
3. L-Dopa therapy for 1 yr or longer (average dose: 4 g day^{-1})				
1,172±173 (5) ‡	887±95 (5) §	1,210±109 (5) §	1,210±110 (4) §	407±67 *

Patients on L-dopa received the drug for different periods in daily oral doses ranging from 2 to 6 g. Enzyme activity (mean±s.e.m.) is expressed as nmol carbon dioxide produced per 100 mg of protein per 2 h. (Number of cases in parentheses.) Brain tissue was homogenized in ice-cold isotonic dextrose and incubated for 20 min (37° C) in the presence of phosphate buffer (*p*H 7.0) and 0.6 mM pyridoxal phosphate. L-Glutamic acid was then added (final concentration of 2.5 mM containing 0.4 μCi DL-glutamic acid-^{14}COOH) and after 2 h the reaction was terminated by addition of acid. The evolved carbon dioxide was trapped in hyamine hydroxide and then assayed for radioactivity.

* Significantly different from group *A* ($P<0.01$). † Significantly different from group *A* ($P<0.001$).

‡ Significantly different from group *B*, 2 ($P<0.05$). § Significantly different from group *B*, 2 ($P<0.001$).

Human or rat brains were dissected and stored on dry ice as previously described[6]. Human material was obtained at autopsy from either control (no known neurological disorder) or Parkinsonian patients. Nine Parkinsonian patients received L-dopa until they died; two others did not receive L-dopa therapy. The patient groups were well matched with respect to age (means: control=68 yr; Parkinsonian=67 yr), sex (controls: ten males and three females; Parkinsonian: nine males and two females) and interval between death and freezing of the tissue (4–21 h for the controls, and 4–17 h for the Parkinsonian patients). None of these parameters had any apparent influence on GAD activity[4].

For the animal experiments, male albino Wistar rats (250–275 g) were fed an aqueous suspension of L-dopa by way of a stomach tube on the following regimen: days 1–37, 50 mg per rat per day; days 38–88, 100 mg per rat per day; days 89–109, 2×100 mg per rat per day. Controls were fed the suspension fluid (water) according to the same schedule. Animals were killed at days 37, 88 and 109. The striata were immediately removed and frozen.

GAD activity was estimated as described for L-dopa decarboxylase[6]. In brief, brain tissue was homogenized in ice-cold isotonic dextrose and incubated at *p*H 7.0 (37° C, 60 min) in the presence of pyridoxal phosphate (0.6 mM) and L-glutamic acid (2.5 mM containing 0.4 μCi DL-glutamic acid-1-^{14}C). The carbon dioxide formed was trapped in hyamine hydroxide and assayed for radioactivity. Blanks contained *p*-bromo-m-hydroxybenzyloxyamine, a potent inactivator of pyridoxal phosphate[7].

The GAD activity in the striatum and substantia nigra of the non-dopa treated Parkinsonian patients was less than 50% of the control mean (Table 1), in agreement with a previous report[1]. In other brain regions GAD activity was within the range of the control values. In the brains of patients who received L-dopa for 8 months or less, GAD activity was significantly lower than in the controls (Table 1). In the caudate nucleus, putamen, substantia nigra, and globus pallidus of patients who received L-dopa continuously for 1 yr or longer, however, GAD activity was within the range of the controls, being significantly greater than that of patients treated for a shorter time. The GAD activity in other brain regions was only slightly higher in patients with the more prolonged L-dopa treatment.

In the rats treated chronically with oral L-dopa, after administration of L-dopa for 37 and 38 days the striatal GAD activity was not different from control (Table 2). After 109 days, however, striatal GAD activity was significantly greater than in either those rats which had received lower doses of L-dopa for a shorter period, or the controls. Thus, it was possible to replicate, in controlled laboratory conditions, the increase in striatal GAD activity observed in Parkinsonian patients chronically treated with L-dopa.

The GAD activity of the substantia nigra and globus pallidus, but not striatum, in Parkinsonism was reported to be subnormal compared with the frontal cortex[8,9]. Absolute GAD activity was, however, decreased throughout the brain compared with controls. Moreover, there was no indication of whether or not L-dopa had been administered. We find that in Parkinson's disease there is a definite decrease in the activity of striatal GAD and that its amelioration by L-dopa therapy is dependent upon the daily dose of L-dopa and the duration of its administration. This implies that the striatal GAD-L-dopa relationship depends on the concentration of dopamine present. In fact, in rats the increase in striatal GAD induced by L-dopa was dose-dependent. In addition, the L-dopa-treated patients with highest GAD activities were treated for longer (1 year or longer) and received, as a group, a higher dose of L-dopa (Table 1). It has also been shown that the striatal dopamine concentration of chronically L-dopa treated patients is markedly higher than in non-dopa treated patients[5,12,13]. Correspondingly, the more chronically treated group of Parkinsonian patients had an average concentration of striatal dopamine (caudate nucleus: 1.6 μg g^{-1}) distinctly higher than in the

Table 2 Effect of Chronic Oral L-Dopa Administration on the GAD Activity of the Rat Striatum

Duration of L-dopa administration (days)	L-Glutamic acid decarboxylase activity		
	Mean±s.e.m.	No. of animals	% of control
Control	7,399±950	7	100.0
37 days	7,360±853	7	99.5
88 days	7,012	2	94.8
109 days	11,310±839	5	152.8 * †

Rats were fed an L-dopa suspension (on the regimen described in detail in the text) by means of a metal stomach tube at 1600 h (days 1–88) or at 0800 and 2000 h (days 89–109). Enzymic activity expressed as nmol carbon dioxide produced per 100 mg of protein per 2 h. Age-matched sham-treated control animals were killed with each experimental group and GAD values did not differ between groups; hence all control values were combined. Assay conditions as in Table 1. * Significantly different from controls, $P<0.002$. † Significantly different from 37+88 day groups, $P<0.005$.

group treated for 8 months or less (caudate nucleus: 0.7 $\mu g\ g^{-1}$).

What is the mechanism of the L-dopa-induced increase in GAD activity? Although large diencephalic lesions in experimental animals decrease GAD activity in the substantia nigra and globus pallidus[8,10], no such lesions are apparent in Parkinson's disease[11]. Thus the decrease in GAD activity in Parkinson's disease may be secondary to the severe degeneration of the nigrostriatal dopamine pathway. These GAD-containing neurones could be under a continuous "trophic" influence of the dopaminergic system; degeneration of the dopaminergic pathway might then result in a biochemical "atrophy" of the GAD neurones. Replenishment of striatal dopamine by L-dopa[4,14] possibly reverses the biochemical atrophy of the GAD-containing neurones, a process that may well require prolonged administration of L-dopa.

Dopamine might act by removing a repression of GAD synthesis. This is an attractive but possibly incomplete explanation because of the long latency before striatal GAD activity increases. Relatively high concentrations of striatal dopamine may be needed, a process requiring a considerable time. This is supported by the lengthy period of L-dopa administration required to increase striatal GAD activity in rat brain.

An alternative hypothesis is an enhanced synthesis of GAD apoenzyme in compensation for decreased cofactor availability as a result of Schiff base formation with L-dopa[15,16]. Such an *in vivo* formation of Schiff base would not be reflected *in vitro* where an optimal concentration of pyridoxal phosphate is added. Signs of vitamin B_6 deficiency are not, however, apparent during chronic L-dopa therapy, making this a rather unlikely hypothesis.

The increase in striatal GAD activity during chronic L-dopa therapy may have an important clinical correlate as it conspicuously parallels the ameliorative effect of L-dopa on Parkinsonian tremor (which may take from several weeks to months to develop[17]). So far the anti-tremor effect of L-dopa has been without any known neurochemical correlate; our observations suggest that a GABA-containing neurone system may be involved. In this context, a new GABA-like compound has recently been reported to exert a specific anti-tremor effect in Parkinsonian patients[18].

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*p*H-Dependent Changes in Composition of Carcinoembryonic Antigen

Carcinoembryonic antigens (CEA) are present in perchloric acid-soluble extracts of carcinoma of the gastrointestinal tract, particularly of the colon and rectum[1]. I report here some observations suggesting that CEA, as present in perchloric acid extracts of carcinomata, is an aggregate of cationic protein and metachromatic material.

Tissue was homogenized in five to ten volumes of saline, an equal volume of 1.2 M perchloric acid added, the mixture centrifuged and the supernatant neutralized with 1 N NaOH; all procedures were carried out at 4° C and with vigorous stirring. Extracts were concentrated to minimal volumes in an ultrafiltration cell using a UM-10 'Diaflo' filter (Amicon Corporation, Lexington, Massachusetts). Three rabbits were given thirteen weekly injections of pooled extracts from nine carcinomata of the colon or rectum, initially combined with Freund's complete adjuvant and then with incomplete adjuvant. Thirty millilitres of pooled antiserum was absorbed with perchloric acid extracts of normal colon and rectum, a mucoprotein fraction from pooled human serum and fractions of human serum. The large and small intestine were obtained within 12 h of death from two subjects (one dying from a gunshot wound, the other from a coronary occlusion), the mucosa and the remainder of the bowel wall were extracted separately and the extracts concentrated to 7.6 ml and 4.2 ml respectively. Mucosa extract (5.1 ml) and 2.3 ml of the muscle coat extract were added to the 30 ml of antiserum. Two serum mucoprotein fractions were prepared from 1,250 ml and 670 ml of human serum by adding an equal volume of 0.4 M perchloric acid, centrifuging and neutralizing the supernatant with 0.5 N NaOH; each was concentrated to approximately 10 ml, and 2.6 ml and 3.4 ml respectively were added to the antiserum. Seven fractions of human serum obtained by gel and ion exchange chromatography were added in amounts ranging from 0.1–17.6 mg of protein ml^{-1} of antiserum.

By Ouchterlony double diffusion, the absorbed antiserum revealed two antigens in extracts of eighteen surgical specimens of carcinomata of the colon or rectum (Fig. 1*A*). One of these antigens was identified as CEA by an antiserum obtained from Dr P. Gold, Montreal General Hospital (Fig 1*A*, *B*). The second antigen has antigenic sites in common with CEA (Fig. 1*C*) and will be called Ca-2. Extracts of macroscopically normal intestine from fourteen of the surgical specimens gave precipitin lines that merged with the CEA and Ca-2 lines given by the corresponding tumour extract. The mucosa extract from the two non-cancerous subjects also gave two very faint lines which merged with the two lines given by an extract of rectal carcinoma. When a portion of the antiserum was further absorbed with an extract of a rectal carcinoma it no longer reacted with any of the above extracts. Other workers have detected CEA in perchloric acid extracts of non-cancerous colon[2].

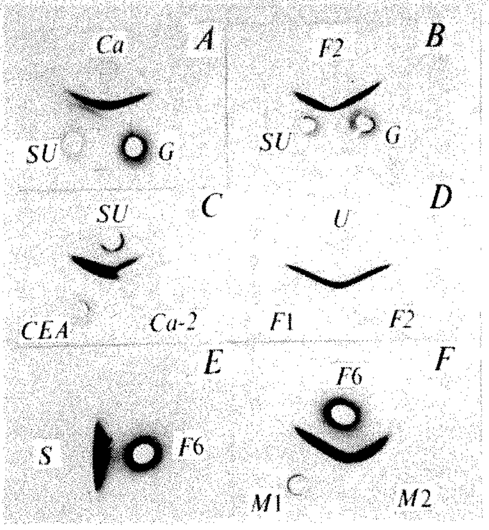

Fig. 1 Ouchterlony double diffusion reactions in 1% agarose. For *A*, barbital buffer[16] was used. For the other plates, 2% agarose was mixed with an equal volume of the Tris-HCl buffer (see text). *SU*, Absorbed antiserum to colonic carcinoma; *G*, anti-CEA (goat 81); *U*, unabsorbed antiserum to colonic carcinoma. *A*: *Ca*, Concentrated extract of a colonic carcinoma. The Ca-2 antigen (see text and *C* below) is indicated by the faint line between the CEA precipitin line and antibody well. *B*: *F2*, fraction 2, Fig. 3*B*. *C*: *CEA*, CEA preparation shown in Fig. 5*A*; *Ca-2*, the *Ca-2* antigen isolated from the same twenty-four carcinomata as CEA and by the same procedures. On gel chromatography *Ca-2* was eluted after CEA but was eluted first during chromatography on DEAE 'Sephadex'. During chromatography on SE 'Sephadex' and DEAE 'Sephadex' at *p*H 2.2, *Ca-2* partly dissociated into a cationic protein and metachromatic material as described for CEA in Fig. 5*B* and *D*. Note the spur given by the CEA precipitin line. *D*: *F1* and *F2*, fractions 1 and 2, Fig. 5*D*. An identical reaction was given by these two fractions and the absorbed antiserum. *E*: *F6*, fraction 6, Fig. 3*C*; *M1* and *M2*, two fractions isolated from the same extract as CEA and which contain metachromatic material. *M1* also contained a small amount of material staining with coomassie blue, but *M2* contained metachromatic material only. These spurious precipitin lines persisted even after washing the slides in 0.15 M NaCl for 4 d.

Pooled extracts from twenty-four carcinomata were chromatographed on a 60×490 mm column of 'Sephadex' G100 (Pharmacia, Uppsala) using a 0.05 M Tris-HCl buffer, *p*H 7.2, containing 0.5 M NaCl. Fractions were concentrated by ultrafiltration with a UM-10 'Diaflo' filter and chromatographed on a 35×120 mm column of DEAE 'Sephadex' equilibrated with 0.0075 M phosphate, *p*H 7.2. Proteins were eluted with a continuous NaCl gradient established by 0.01 M phosphate buffer, *p*H 7.2, containing 0.2 M NaCl. Fractions containing CEA were pooled and chromatographed on 'Sephadex' G200 (Fig. 2*A*) and then on 'Sepharose' 6B (Fig. 2*B*). The presence of more than one CEA peak after chromatography on 'Sephadex' G200 has been reported by other workers[3,4]. 'Sepharose' 4B has also been used to isolate CEA and is eluted from it with a percentage column volume of 70% to 80%[3,4].

During the isolation, concentrated fractions were tested by (*a*) Ouchterlony double diffusion and immunoelectrophoresis using both the absorbed and unabsorbed antisera and (*b*) electrophoresis followed by staining with coomassie blue for protein, with toluidine blue for metachromatic substances[5], or by staining for the periodic acid Schiff (PAS) reaction for carbohydrate[5]. CEA eluted from the 'Sepharose' 6B column contained no material staining metachromatically but contained a trace amount of a PAS positive substance migrating to the cathode (Fig. 3*A*). Antisera (Behringwerke AG) to six serum proteins soluble in perchloric acid (namely, α_1-antitrypsin, α_2-HS-glycoprotein, Zn-α_2-glycoprotein, β_2-glycoprotein, haptoglobin and haemopexin) did not react with this CEA preparation in Ouchterlony double diffusion. Immunoelectrophoresis revealed diffuse material and one precipitin arc merging with the CEA arc (Fig. 3*A*). Preparative electrophoresis in agarose failed to remove this additional material (Fig. 3*B*).

Fraction 2 (Fig. 3*B*), obtained by preparative electrophoresis, was chromatographed on DEAE 'Sephadex' at *p*H 5.7—that is, at a lower *p*H than that used during isolation of CEA from the cancer extracts. On immunoelectrophoresis the shape and position of the CEA arcs varied between different fractions eluted and from that of the starting material (Fig. 3*C*). Two fractions contained a cationic protein which had no specificity for CEA and which stained much more intensely with coomassie blue than CEA (Fig. 3*C*). Dissociation of CEA by a comparatively minor change in *p*H (from 7.2 to 5.7) suggests that the peculiar immunoelectrophoretic pattern (Fig. 3*A*, *B*) resulted from dissociation caused by the voltage applied during electrophoresis[6].

When the cationic protein (fraction 6, Fig. 3*C*) was allowed to diffuse against other proteins in a manner analogous to Ouchterlony double diffusion, precipitates resembling immune precipitin lines were formed even in the presence of 0.25 M NaCl (Fig. 1*E*, *F*). When the three fractions retaining CEA specificity (fractions 2, 3 and 4, Fig. 3*C*) were pooled and tested in the same manner as above, this pooled material also gave a spurious precipitin line when allowed to diffuse against the metachromatic material but did not form a precipitate with the serum mucoprotein fraction.

Fraction 2 (*F2*, Fig. 2*A*), eluted after the main CEA peak on 'Sephadex' G200 chromatography, and which contained both CEA and the Ca-2 antigen, was equilibrated with 0.01 M glycine-HCl buffer, *p*H 2.2, in an ultrafiltration cell with a UM-2 'Diaflo' filter. Filtrate from this cell gave a precipitate with 8% phosphotungstic acid solution. The precipitable material in this filtrate was restrained by a UM-05 'Diaflo' filter. When material retained by the UM-2 filter (see above)

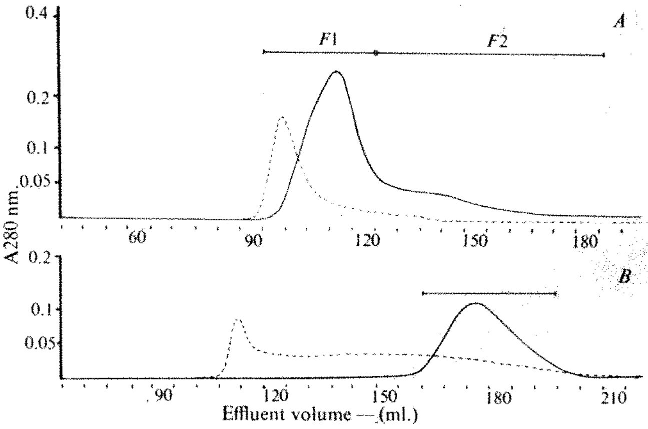

Fig. 2 Gel chromatography of CEA fractions. A 0.05 M Tris-HCl buffer, *p*H 7.2, containing 0.5 M NaCl, 0.02% sodium azide and 1% butanol was used for elution in each case. Eluate was monitored at 280 nm. Dimensions of the packed columns were 25×531 mm for the 'Sephadex' G200 and 25×541 mm for the 'Sepharose' 6B column. Six millilitre fractions were collected at a flow rate of 14.4 ml h^{-1}. Void volumes were obtained by chromatography of 'Blue Dextran' 2000 (Pharmacia, Uppsala). ——, Recording given by CEA; - - -, recording given by 'Blue Dextran'. *A*, Chromatography on 'Sephadex' G200 of the CEA preparation described in the text. The partition coefficient and % column volume for the main CEA peak (*F1*) were 0.08 and 35% respectively. Fractions under the line marked *F1* were concentrated with a UM-10 'Diaflo' filter and chromatographed on 'Sepharose' 6B (see *B*). The CEA in *F1* stained blue with toluidine blue but showed no metachromasia. Fractions under the line marked *F2* were pooled and concentrated with a UM-10 filter (see Fig. 4*A*). *B*, Chromatography on 'Sepharose' 6B of fraction *F1* in *A* above. CEA was eluted with a partition coefficient of 0.38 or a percentage column volume of 62%. Fractions under the horizontal line were pooled and concentrated with a UM-10 filter (see Fig. 3*A*).

was chromatographed on DEAE 'Sephadex' at pH 2.2, the greater part of the starting material was converted to proteins migrating to the cathode, some of which retained specificity for CEA but with weaker PAS reactions than the starting material (Fig. 4*A*, *B*).

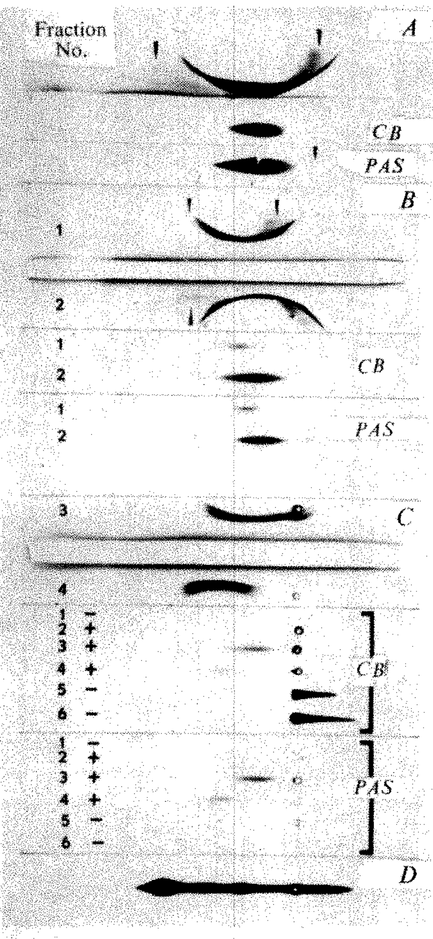

Fig. 3 Immunoelectrophoretic and electrophoretic patterns of concentrated fractions of CEA. Electrophoresis was carried out on glass slides (25×76 mm), using 1% agarose (General Biochemicals, Ohio) and a discontinuous barbital buffer, pH 8.6 (ref. 16), at a constant current of 5 mA per slide for 25 min. Unabsorbed antiserum to colonic carcinoma was used for all the immunoelectrophoretic patterns. Immunodiffusion was carried out for 48 h at 37° C. For electrophoretic patterns, the slides were fixed in methanol, dried and stained with coomassie blue (CB) or by the periodic acid Schiff (PAS) reaction for carbohydrate. Slides were stained for 15 min in 0.2% coomassie blue in methanol-water-acetic acid (50–40–10), and washed in methanol-water-acetic acid (50–145–5). Arrows indicate unusual features of the patterns and the contaminant detected by the PAS reaction. The detection of CEA by Ouchterlony double diffusion is recorded as positive (+) or negative (−). For all slides illustrated, the anode was on the left side. *A*, The concentrated CEA fraction obtained by chromatography on 'Sepharose' 6B (Fig. 2*B*). *B*, Two of three fractions obtained from the CEA fraction shown in *A* above by preparative electrophoresis in agarose. Electrophoresis was carried out as above, the agarose cut across to separate the leading, intermediate and trailing segments of the CEA region, and the separated portions eluted with 0.05 M Tris-HCl buffer, pH 7.2, containing 0.5 M NaCl. *C*, Fractions obtained by ion exchange chromatography of fraction 2 in *B* above. A column (25×340 mm) of DEAE 'Sephadex' was equilibrated with 0.005 M phosphate buffer, pH 5.7, as starting buffer. Fractions 1–4 were eluted by a continuous NaCl gradient. After gradient elution, the column was washed with (*a*) 1.0 M NaCl in phosphate buffer (fraction 5), and (*b*) 1.0 M NaCl in 0.01 N HCl (fraction 6). Immunoelectrophoresis with absorbed antiserum to colonic carcinoma gave the same patterns as those shown here for fractions 3 and 4 with the unabsorbed antiserum. *D*, Electrophoretic pattern of serum stained with coomassie blue.

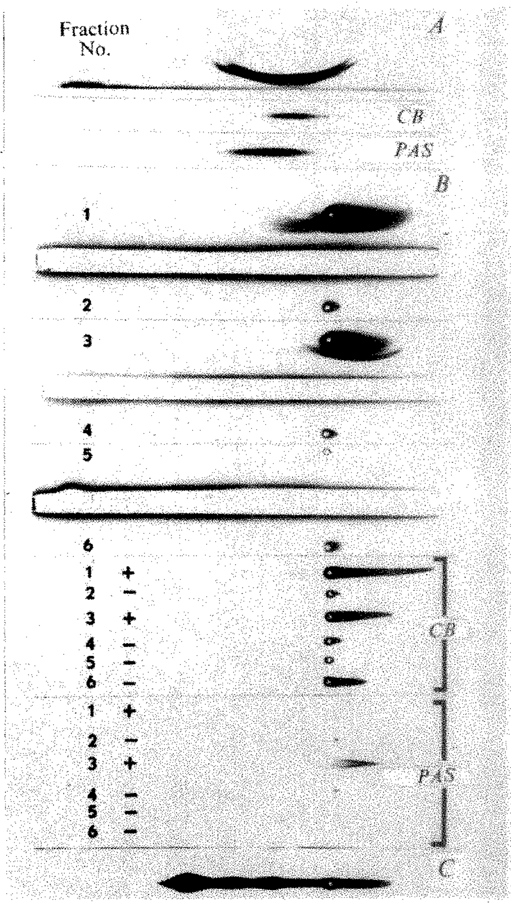

Fig. 4 Immunoelectrophoretic and electrophoretic patterns of concentrated fractions which contain both CEA and the Ca-2 antigen. Electrophoresis and immunoelectrophoresis were carried out as described in Fig. 3. *CB*, Coomassie blue stain; PAS, periodic acid Schiff stain for carbohydrate. The detection of Ca-2 and CEA by Ouchterlony double diffusion is recorded as positive (+) or negative (−). *A*, A fraction isolated by chromatography on 'Sephadex' G200 (*F*2, Fig. 2*A*). The precipitin arc due to the Ca-2 antigen is nearest the antibody trough, and the CEA arc lies above and parallel to it. *B*, Fractions isolated from *F*2 shown in *A* above by ion exchange chromatography on a column (25×355) of DEAE 'Sephadex' which was equilibrated with 0.01 M glycine-HCl buffer, pH 2.2. Material in fraction 1 passed through the column without being bound to the DEAE 'Sephadex'. Fractions 2–5 were eluted with a continuous NaCl gradient produced by 0.5 M NaCl in the same glycine buffer. Fraction 6 was then eluted from the column with 1 M NaCl in 0.01 M HCl. Note that for fraction 1, and to a lesser extent for fraction 3, continuous arcs run from the slow α_2 region through to the γ region of the immunoelectrophoretic patterns. Note also the loss of PAS reactivity in fractions 1 and 3 compared to the starting material. *C*, Electrophoretic pattern of serum stained with coomassie blue.

Fractions 2, 3 and 4, Fig. 3*C*, were pooled and chromatographed on a cation exchanger (SE 'Sephadex') at pH 2.2. The immunoelectrophoretic arc of the eluted CEA differed from that of the starting material while all the fractions eluted contained negatively charged, metachromatic material (Fig. 5*A*–*C*).

Finally, fraction 3 (Fig. 5*B*) from the chromatography on SE 'Sephadex' was chromatographed on DEAE 'Sephadex' at pH 2.2 (Fig. 5*D*). Immunoelectrophoresis revealed changes in the shape and position of the CEA arc from that of the starting material (fraction 2, Fig. 5*D*). A fraction eluted after CEA contained metachromatic material (fraction 3, Fig. 5*D*).

It is not clear whether any CEA determinants are covalently linked to the cationic protein. A basic protein that has been isolated from bovine spinal cord by extraction with HCl at pH 1.7[7] acts as a natural receptor for N-acetyl-galactos-

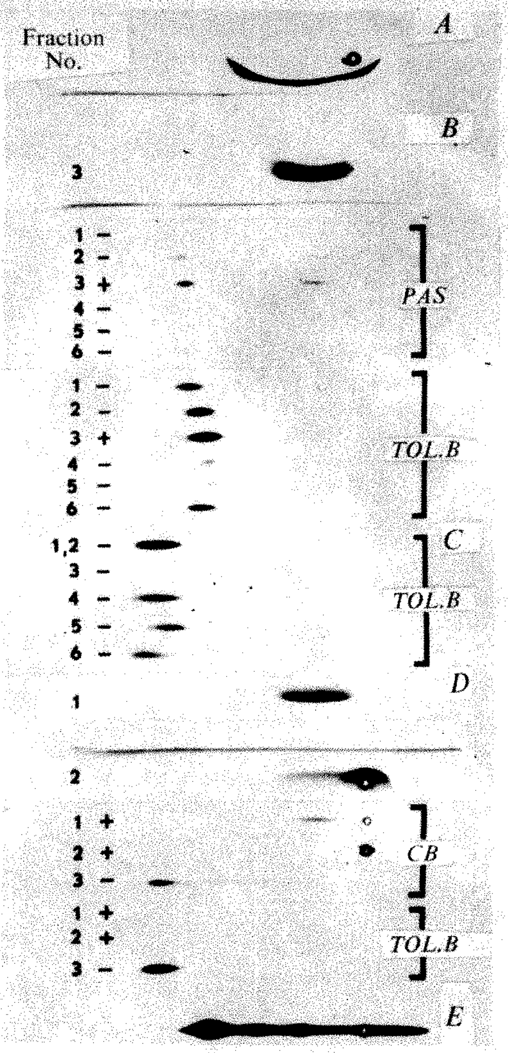

Fig. 5 Immunoelectrophoretic and electrophoretic patterns of concentrated fractions of CEA carried out as described in Fig. 3. The immunoelectrophoretic patterns shown were produced by the unabsorbed antiserum to colonic carcinoma; identical patterns were given by the absorbed antiserum. The detection of CEA by Ouchterlony double diffusion is recorded as positive (+) or negative (−). PAS, Periodic acid Schiff reaction for carbohydrate; TOL B, toluidine blue stain for metachromatic substances[5]; CB, coomassie blue stain. *A*, Pooled CEA fractions (fractions 2, 3 and 4, Fig. 3*C*). *B*, Fractions obtained by ion exchange chromatography on SE 'Sephadex' of the CEA shown in *A* above. A column (25 × 280 mm) of SE 'Sephadex' was equilibrated with 0.025 M glycine-HCl buffer, pH 2.2, as starting buffer. Fraction 1 passed through without being bound. Fractions 2–5 were eluted by a continuous NaCl gradient produced by 1.0 M NaCl in the same glycine buffer. Fraction 6 was then eluted with 0.1 M glycine-NaOH buffer, pH 9.6, containing 1.0 M NaCl. Fractions were concentrated with a UM-10 'Diaflo' filter. The rapidly migrating material present in all six fractions was strongly metachromatic and faintly PAS positive. *C*, Material present in the UM-10 filtrates of the six fractions shown in *B* above and which was retained by a UM-05 filter. The UM-10 filtrates from fractions 1 and 2 in *B* above were pooled, while the remaining four UM-10 filtrates were concentrated with the UM-05 filter individually. The retained material was strongly metachromatic and faintly PAS positive. *D*, Fractions obtained by chromatography on DEAE 'Sephadex' of fraction 3 in *B* above. A column (15 × 200 mm) of DEAE 'Sephadex' was equilibrated with 0.025 M glycine-HCl buffer, pH 2.2. Fraction 1 was eluted in the starting buffer and fraction 2 by a continuous NaCl gradient using 0.6 M NaCl in the same glycine buffer. Fraction 3 was then eluted with 1 M NaCl in 0.01 N HCl. Fractions were concentrated with a UM-10 'Diaflo' filter. No difference could be detected between the CEA in fractions 1 and 2 by Ouchterlony double diffusion (Fig. 1*D*). The CEA in fractions 1 and 2 and the metachromatic material in fraction 3 were faintly PAS positive. When the UM-10 filtrates from the three fractions were concentrated separately with a UM-05 filter, metachromatic material which was faintly PAS positive was found only in the retentate of fraction 3. *E*, Electrophoretic pattern of serum stained with coomassie blue.

amine in the presence of UDP-N-acetyl-galactosamine and a polypeptidyl: N-acetyl-galactosamine transferase from bovine submaxillary glands[8]. N-Acetyl-galactosamine is present in blood group A substance, and although CEA contains no N-acetyl-galactosamine it does show blood group A-like activity[9,10]. N-Acetyl-glucosamine is a characteristic constituent of CEA[9]. Perchloric acid extracts of colonic carcinoma contain cationic proteins that do not bind to DEAE 'Sephadex' (unpublished data). That cationic proteins may also be present in homogenates of colonic carcinomata is suggested by the observation that human, malignant cell lines have on their surface[11] an antigen that is immunologically related to basic proteins which can be extracted from these cell lines and from human brain and malignant tissue by treatment with HCl at pH 2.5[11–13]. CEA specificity could therefore be formed during the homogenization of carcinomata by glycosyl transferases coupling carbohydrate residues to sites that are not exposed in normal tissues. Again, it may be pertinent to draw attention to the observation that low molecular weight (about 5,000) glycopeptides form a major fraction of cell membranes and can be extracted with either sodium dodecyl sulphate or formic acid[14]. Polymerization is a characteristic of these glycopeptides and lyophilization results in aggregates that cannot be readily dissociated[14]. CEA is a component of the cell surface[15].

Because of the unusual characteristic of the cationic protein present in CEA, it was considered that these preliminary observations might be of particular interest to workers using CEA preparations in radioimmunoassay and other immunological tests.

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Receptor for Monomeric IgM on Guinea-pig Splenic Macrophages

It is now well established that an optimal immune response resulting in the production of antibody to most antigens requires interaction between three distinct cell types: T (thymus-derived) and B (thymus-independent) lymphocytes[1], and macrophages[2]. Studies to elucidate the mechanism of interaction or collaboration have implicated soluble factors released by T cells which include both non-specific components[3-6] and antigen-specific components[6-9] which are bound by macrophages[8,9]. IgM has been demonstrated in T cell membranes[10,11] in monomeric form[11]. Antigen binding by T cells, as judged by rosette formation[12] or specific helper function at low doses of antigen[13], can be inhibited by antibody to IgM determinants. Evidence suggests that the specific collaborative factor released by T cells consists of antigen complexed with monomeric IgM, which becomes bound to the surface of macrophage-like cells (refs. 9, 14 and 15 and E-P. Rieber and G. Reithmuller, submitted for publication). Macrophage binding of antigen specific factors released by T cells has also been shown in studies on the killing of allogeneic tumour cells *in vitro*[16]. Here I show that a receptor for monomeric IgM is present on macrophages from guinea-pig spleen. Pentameric (19S) IgM is not bound by these cells to any significant extent. Splenic macrophages were defined by the following characteristics: (1) rapid uptake of neutral red stain; (2) ingestion of carbonyl iron particles; (3) presence of a receptor for the Fc portion of guinea-pig γG_2; (4) uptake *in vivo* of alum precipitated bovine serum albumin (BSA).

My data, summarized in Tables 1 and 2, show that approximately 1% of spleen cells possess a receptor which is specific for the Fc portion of IgG_2 but not for IgM. Such a receptor has been shown to be characteristic of guinea-pig alveolar[17] and peritoneal macrophages[17,18]. Rosette forming cells (RFCs) were also found to stain rapidly with neutral red (Table 2). Approximately 50% of stained cells had the appearance of macrophages, the remainder consisted of monocytes and Kurloff cells[19]. Macrophages together with some monocytes were selectively depleted using carbonyl iron and magnetic precipitation (Table 1). The uptake of alum precipitated BSA *in vivo* was also found to be a property of receptor-bearing macrophages (Table 2).

Results of rosette formation are presented in Table 3,

Table 1 Rosette Formation on Normal and Macrophage-depleted Spleen Cells using IgG* and IgM †-sensitized Red Cells

Rosette forming cells per 10^3 spleen cells ‡		
IgM-coated SRBCs plus normal spleen cells	IgG-coated SRBCs plus normal spleen cells	IgG-coated SRBCs plus macrophage depleted spleen cells
1.0	15.0	6.0
1.5	12.0	4.5
1.3	8.5	5.5
0.6	15.5	5.5

* Concentration, 0.25 mg ml^{-1}.
† Concentration, 0.5 mg ml^{-1}.
‡ Mean sample size = 3.5×10^3 spleen cells.

Splenic tissue was minced and irrigated with Hanks balanced salt solution (Hanks BSS) *p*H 7.2. Cells were separated from tissue fragments by differential centrifugation and freed of erythrocytes by differential hypotonic lysis and agglutination.

Splenic cells were subjected to a rosette formation procedure using sheep red blood cells (SRBCs) sensitized with guinea-pig anti-SRBC antibody of either the γG or γM class (twice separated on 'Sephadex' G200). The degree of coating with 19S and 7S antibody was comparable as judged by haemagglutination using rabbit anti-guinea-pig serum.

0.1 ml of a 50% suspension of SRBCs was incubated with 0.5 ml of antibody at the concentration stated, in Hanks BSS *p*H 7.2 for 90 min at 37° C. The cells were then washed twice and resuspended as a 20% suspension. Rosette formation was carried out by mixing 0.1 ml of this suspension with 0.1 ml of a suspension of nucleated spleen cells containing 10^7 cells. The mixture was centrifuged at 220*g* for 5 min at room temperature and resuspended by gentle aspiration in a Pasteur capillary pipette repeated twelve times. The cells were examined under phase contrast illumination at × 600. Nucleated cells completely surrounded by ten or more SRBCs were scored as rosettes.

Table 2 Representative Table: Characterization of Receptor-specificity and Receptor-bearing Cells

Positive cells per 10^3 spleen cells					
IgG rosettes Pretreatment ‡		Neutral red staining * Pretreatment		Antigen uptake † Pretreatment	
None	8.2	None	7.0	None	6.0
IgG_1	5.9	None	16.0	None	5.6
IgG_2	0.7	None	10.0	None	5.3
Fab	8.4	None	11.2		
Fc	0.7				

* Spleen cells at a concentration of 5×10^7 cells ml^{-1} were stained in a 0.002% solution of neutral red. At room temperature receptor bearing cells stained rapidly (2–5 min).
† 0.5 ml of fluorescein-conjugated BSA at 20 mg ml^{-1} precipitated on alum was injected intravenously and spleen cells were extracted after 2 h. Following rosette formation with IgG-sensitized SRBCs, RFCs were examined for fluorescence in ultraviolet illumination.
‡ Spleen cells at a concentration of 7.5×10^7 cells ml^{-1} were incubated with IgG subclass preparations at 6 mg ml^{-1} or IgG_2 molecular fragments at 1 mg ml^{-1} for 30 min at room temperature. The cells were centrifuged down and resuspended in fresh Hanks BSS.

Table 3 Rosette Formation on Normal and Macrophage-depleted Spleen Cells using SRBCs Sensitized with Pentameric (19S) IgM or Monomeric (7S) IgM

Rosette forming cells per 10^3 spleen cells *			
SRBCs coated with 19S IgM at a concentration of:		SRBCs coated with monomeric IgM at a concentration of:	
1 mg ml^{-1}	0.5 mg ml^{-1}	0.5 mg ml^{-1}	0.5 mg ml^{-1}
Normal spleen cells	Normal spleen cells	Normal spleen cells	Macrophage depleted spleen cells
3.0	1.0	8.1	3.2
2.75	1.5	8.0	3.2
2.2	1.7	4.5	2.9
2.5	1.5	7.6	3.4
		7.8	3.0

* Mean sample size = 4×10^3.

Monomeric IgM was prepared by reduction and alkylation of IgM obtained from guinea-pig and anti-SRBC antisera. Dithiothreitol (DTT) at a concentration of 0.7 mM was incubated with IgM at 2.5 mg ml^{-1} in 100 mM Tris HCl *p*H 8.0 for 1 h at room temperature. Reduction was stopped by addition of a four-fold molar excess of iodoacetamide and the solution was dialysed against Hanks BSS *p*H 7.2 for 18 h. The optimal concentration of DTT in this procedure was determined using a range of DTT solutions (0.05–1.5 mM). Thus, after an 18 h dialysis the reduction products were analysed by polyacrylamide disc electrophoresis in the presence of sodium dodecyl sulphate[25]. The major products obtained using 0.5–1.0 mM concentrations of DTT were monomeric IgM (IgMs) and a half molecule consisting of one heavy and one light chain (H–L fragment). Traces of trimer and dimer were detectable using 0.6 mM DTT.

SRBCs were specifically coated with monomeric IgM and possibly H–L fragments[20] by incubation for 90 min at 37° C in a solution containing reduction products at a concentration of approximately 1 mg ml^{-1}. Red cells were then washed once in a 20-fold volume of Hanks BSS and resuspended as a 20% suspension. Rosette formation on spleen cells using SRBCs sensitized in this way was compared with rosette formation using SRBCs sensitized with pentameric (19S) IgM. Two controls were adopted, one using SRBCs coated with IgM at 0.5 mg ml^{-1} (the approximate concentration of monomeric IgM in the sensitizing solution) and the other using IgM at 1 mg ml^{-1} (the concentration of the total reduction products: IgMs + H–L).

Magnetic depletion of macrophages together with some monocytes was achieved by incubating spleen cells at a concentration of 15–20×10^6 cells ml^{-1} with carbonyl iron powder (type SF, GAF Gt. Britain Ltd, Manchester) at a concentration of 3 mg ml^{-1} in Hanks BSS *p*H 7.2, for 30 min at 37° C with intermittent mixing. Incubation was carried out in tubes coated with dimethyldichlorosilane and macrophages were precipitated by repeated exposure of the suspension to a magnetic field.

Significant binding was found only in the monomeric IgM preparation. RFC were identified as macrophages by magnetic depletion following carbonyl iron ingestion. Thus macrophages in guinea-pig spleen, previously characterized by the four properties described, also bind monomeric IgM specifically combined with SRBC determinants. Binding occurs in the absence of complement and lymphocytes do not show binding activity; pentameric (19S) IgM is not bound to any significant extent. The receptor may also be present on monocytes which are not depleted by the carbonyl iron procedure.

The hinged nature of pentameric IgM[20,21] may allow attachment of all combining sites to red cell determinants. The ratio of monomeric to pentameric molecules on equivalent areas of red cell surface, however, is unlikely to be greater than 5 : 1 and in antibody excess will be less than this so that it is not an increase in the number of $Fc\mu$ regions per unit surface area which facilitates binding of monomeric IgM.

The receptor for monomeric IgM on splenic macrophages may be involved in the collaborative function of such cells in the immune response[7,14,15]. Feldmann has suggested that the binding by macrophage-like cells of monomeric IgM/antigen complexes released by T cells may facilitate the presentation to B cells of antigenic determinants in an immunological array[9]. The model suggests a possible regulatory effect in T-B cell cooperation in terms of saturation of macrophage receptor sites and could account for antigenic competition at the level of availability of receptors for monomeric IgM[22]. Any equation between reductive monomer and T cell IgM must be made with caution, however[15,23]. Another area in which a macrophage receptor for monomeric IgM might be involved is in the specific cytotoxic arming of macrophages by antigen specific factors released by T cells[16].

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Cocrystallization of Proinsulin and Insulin

THE occurrence of small amounts of proinsulin in crystalline preparations of bovine, porcine, and rat insulin[1–4] and the many similarities in properties of proinsulin and insulin prompted us to consider the possibility that proinsulin cocrystallizes with insulin. Spectral[5] as well as immunological[6] studies have provided evidence that the insulin moiety in proinsulin has a very similar conformation to that of insulin, and proinsulin exhibits the same tendency to form dimers in acidic solution and higher polymers, mainly hexamers, in neutral solutions containing zinc ions[7]. Studies on crystals of proinsulin by Low and coworkers[8] have confirmed the presence of dimers in the unit cells of these crystals. All these observations suggest that the connecting polypeptide segment in proinsulin is oriented away from those surfaces involved in dimer and hexamer formation.

Hexameric structures stabilized by two zinc atoms have been shown by the recent work of Hodgkin and her coworkers[9] to form the unit cell in porcine zinc insulin crystals. The X-ray diffraction studies also have revealed that the carboxyl terminal region of the B chain and the amino terminal region of the A chain are located about 8 Å apart, near the external or peripheral surface of the insulin monomers within the hexamers. These locations would allow

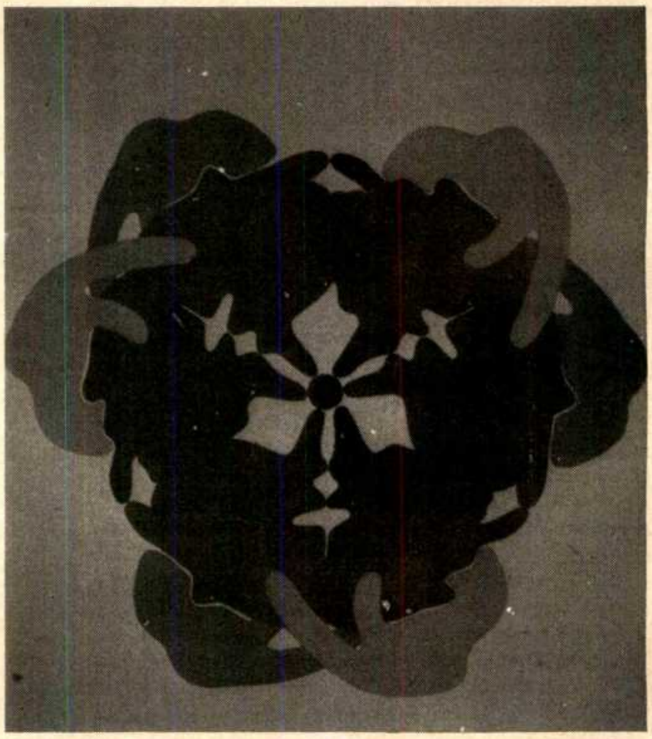

Fig. 1 Hypothetical representation of a proinsulin hexamer viewed along the three-fold axis of the hexamer. The central density represents the two zinc atoms which coordinate to the six histidine side chains at position 10 in the B chain. The densities surrounding the zinc atoms and extending towards them represent the 6 insulin monomers outlined according to the data of Hodgkin and coworkers[9]. The connecting polypeptide is shown in lighter grey around the peripheral portion of the insulin hexamer and the sites of attachment to the insulin chains are indicated by the arms.

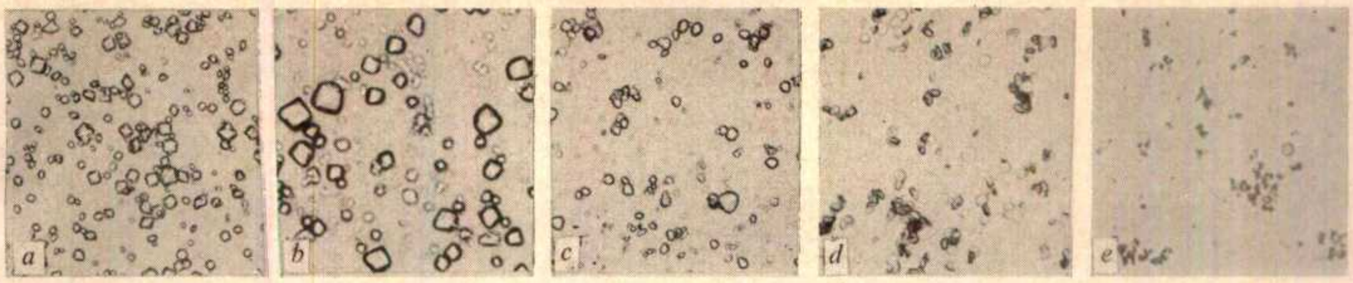

Fig. 2 Photographs of crystals obtained with various proportions of proinsulin and insulin. *a*, Insulin, ×120; *b*, 8% proinsulin, ×120; *c*, 17% proinsulin, ×120; *d*, 33% proinsulin, ×250; *e*, 50% proinsulin, ×250.

the connecting polypeptide to extend into the space overlying those regions of the insulin monomer that are normally exposed to the solvent in solution. Whether the connecting peptide has a defined, three-dimensional arrangement over this region of the insulin moiety of proinsulin is a question that has not yet been resolved, although optical studies have not provided evidence for the existence of defined secondary structure in this part of the molecule. A hypothetical arrangement of a proinsulin hexamer is illustrated in Fig. 1.

If the structure shown in Fig 1 is correct in principle, then it is easy to visualize the replacement of individual proinsulin molecules within such a hexamer with insulin molecules. Conversely, in insulin hexamers, an occasional molecule of proinsulin might replace an insulin monomer. Of course, in the presence of a relatively large proportion of proinsulin ($<5-10\%$), the connecting polypeptides would undoubtedly interfere with the packing of hexamers into crystals. Low proportions of proinsulin might, however, be well tolerated within insulin crystals and this could account for its tendency to be retained through repeated recrystallizations of the insulin.

To test this hypothesis, we attempted crystallization of mixtures of bovine proinsulin and insulin in various proportions corresponding on a molar basis to 0.5, 1.0, 2.0 and 3.0 mol proinsulin/6 mol total of insulin and proinsulin. Crystallization was carried out in 0.095 M sodium citrate buffer (*p*H 6.0) containing 0.03 M NaCl, 0.012 M $ZnCl_2$ and 16% acetone (v/v) at room temperature or at 5° C. Initial protein concentration was 0.33 μmol ml^{-1} in all cases. There was a striking correlation between the amount of proinsulin added to the mixture and the time required for the first visible signs of crystallization. Thus insulin alone began to crystallize within 5 min. With 8% proinsulin crystallization required 20–30 min; with 17%, several hours were required; with 33%, 1–2 days, and with 50% approximately 1 week was required at 5° C before very small crystals began to appear.

Photographs of the crystals are shown in Fig. 2. In the samples containing 8 or 17% proinsulin, rhombohedral crystals were formed but these differed from those of pure insulin in having significantly rounded borders. No normal crystals were observed although there was great variability in size, some being quite large. With 33% proinsulin, the crystals were smaller and in many instances were so distorted that they resembled flattened, round disks suggestive of erythrocytes. Some rounded rhombohedral forms were also evident but no normal crystals could be seen. With 50% proinsulin, very fine crystals were obtained which were either rounded or so small that definite characteristics could not be ascertained. No new crystalline form appeared even after storage for more than 30 days.

The tubes containing the crystals were centrifuged and the mother liquor was separated from the crystals which were washed once with fresh cold crystallization solution. The crystals were then dissolved in Tris-glycine buffer (*p*H 8.6) and aliquots were subjected to polyacrylamide electrophoresis at *p*H 8.9 (Fig. 3). The stained gels were scanned with a Schoeffel Instrument Corp. Model SD-3000 densitometer. By comparison with stained standards of proinsulin and insulin, it could be shown that in each case the proportions of proinsulin and insulin in the crystal corresponded well with those in the initial mixtures without showing any apparent deviation even in the samples containing 33% proinsulin (the 50% crystals were not analysed).

These results confirm the hypothesis that proinsulin can cocrystallize with insulin. Proinsulin itself does not crystallize under these conditions and no indications were seen of the presence of different kinds of crystals in these preparations, so it seems very likely that the composition of the crystals with respect to proinsulin and insulin was relatively uniform throughout. Similarly, the inhibition of crystallization in the mixtures containing a high proportion of proinsulin suggests that the association properties of proinsulin are closely similar to those of insulin. Thus, presumably, mixed dimers and higher polymers form relatively rapidly, but because of the lack of symmetry these tend to pack more slowly into crystals. It is possible that by systematically varying the crystallization conditions, a new mixed crystal form of proinsulin and insulin might be found, and such forms might lend themselves to the elucidation of the three-dimensional structure of proinsulin. The ability of proinsulin to efficiently cocrystallize with insulin provides a convenient and powerful tool for isolating small amounts of proinsulin and related intermediate structures from pancreatic extracts by crystallization with the insulin. This method proved valuable for isolating small amounts of human proinsulin[10].

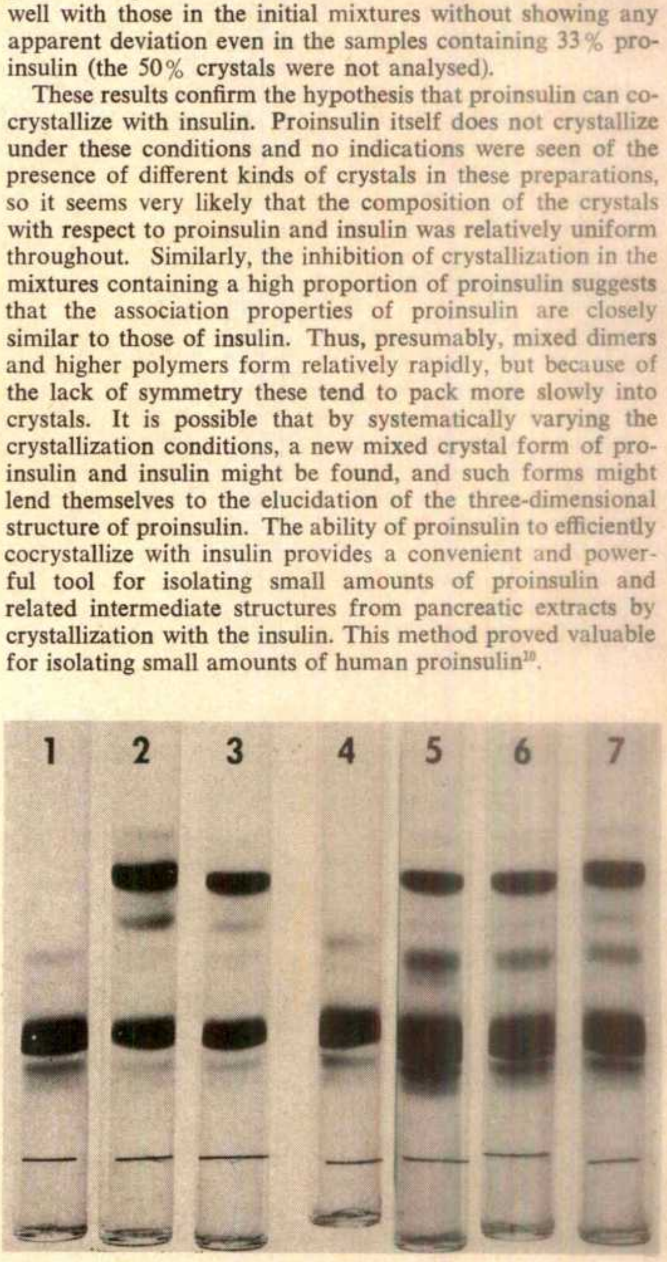

Fig. 3 Photographs of polyacrylamide gel electrophoretograms stained with amido schwartz. Anode is below, proinsulin is the major slow migrating component. The gels were loaded as follows: (1) standard of insulin, 80 µg; (2) standard of insulin, 40 µg, and proinsulin, 60 µg; (3) standard of insulin, 40 µg, and proinsulin, 30 µg; (4) insulin crystals; (5) 8.3% proinsulin crystals (measured value: 9%); (6) 16.7% proinsulin crystals (measured value: 17.5%); (7) 33.3% crystals (measured value: 32%).

It may be anticipated that cocrystallization may occur between many precursor and product peptides, and among other closely related proteins. Moreover, the ability of proinsulin to form hexamers in which the connecting polypeptide is on the external exposed surfaces suggests a possible role for these structures in regulating the conversion of proinsulin to insulin and protecting the newly formed insulin during its biosynthesis within the pancreatic beta cells. Particularly the retarding of crystallization by proinsulin may prevent the premature formation of crystalline granule inclusions[11] before the conversion of most of the proinsulin has occurred.

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Functional Preservation of Canine Heart–Lung Preparations by Perfusion with Pure Haemoglobin Solution

THIS study deals with classic *ex-vivo*, self-perfusing heart–lung preparations, in which the circulating medium is a pure human haemoglobin solution free of stroma. Although this scheme was first devised for preservation for subsequent transplantation, it also provides a convenient method of testing the effects of pharmacological agents on such preparations without interference from the figured elements of the blood for periods up to 6 h.

The haemoglobin solution was prepared by lysis of outdated packed erythrocytes in hyposmolar phosphate buffer, ultracentrifugation, and microfiltration through a series of filters down to a pore size of 0.22 µm. Electrolyte balance similar to that of human plasma was achieved by dialysis through a Kolff twin coil artificial kidney against a standard dialysing fluid adjusted to *p*H 7.4. All procedures but the last were carried out at 4° C[1-4]. Mongrel dogs were heparinized (2 mg kg^{-1}) and killed with an overdose of 'Pentothal'. The thorax of each animal was opened by an incision which split the sternum. With the lungs being ventilated mechanically, the azygos vein, venae cavae, brachiocephalic artery and thoracic aorta were clamped. The subclavian artery was cannulated and connected to a reservoir filled with perfusate at 37° C. The heart beat was restored by gentle massage and defibrillation. The heart–lung preparation, now self-perfusing, was dissected out and transferred to a homoeostatic chamber where it was partially immersed in a solution of balanced electrolytes at 37° C doubling as hydraulic support[5].

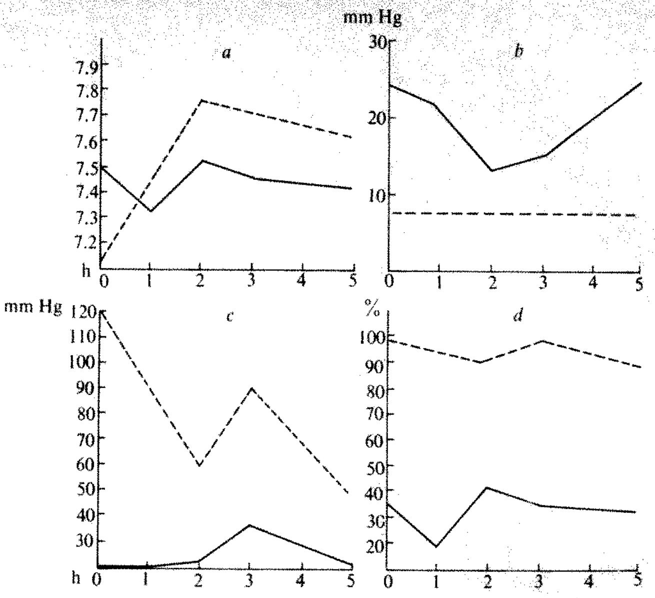

Fig. 1 Variations of *a*, *p*H, *b*, pCO_2, *c*, pO_2, and *d*, haemoglobin oxygen saturation in whole canine blood and human haemoglobin solution during functional preservation of self-perfusing heart–lung preparations. ——, Fresh heparinized whole canine blood; - - -, human haemoglobin solution.

In six experiments, the human haemoglobin solution was used as the perfusate. The blood trapped in the heart and lungs was flushed through the brachiocephalic artery, the haemoglobin solution being, therefore, the only medium within the cardiopulmonary circulation. Controls consisted of six identical preparations perfused with heparinized blood obtained by previous exsanguination of the experimental animal into the reservoir.

In all cases, the pressure in the brachiocephalic artery was monitored continuously and samples of blood or haemoglobin were taken from the vessel at regular intervals for *p*H, pCO_2, pO_2, and haemoglobin oxygen saturation determinations. As progressive seepage from the preparations decreased the volume of the reservoir, blood or haemoglobin solution were added but no nutrients or insulin. Experiments were terminated when the heart went into irreversible fibrillation or when the available supply of blood or haemoglobin was exhausted. Histological examination was performed on all preparations.

"Survival" times for the haemoglobin-perfused preparations ranged from 2 to 6 h (average 3.5), while the blood-perfused controls lasted from 5 to 12 h (average 8.25). Both experimental and control arterial pressures were comparable and remained steady until the end, which was usually preceded by an abrupt fall in pressure.

From Fig. 1, which gives the average values for *p*H, pCO_2, pO_2, and haemoglobin oxygen saturation during the first 5 h in each group, it can be seen that haemoglobin oxygen saturation remained at 90–99% throughout in the experimental group, but dropped within the 30–45% range in the control group. The preparations perfused with human haemoglobin tended to become markedly alkalinic, but when fresh solution was added to the reservoir, there was a sharp drop in *p*H: for instance, in one experiment, from 7.90 to 7.40 (the *p*H of the fresh solution), followed by a rise to a final value of 7.80. A marked rise in pO_2 also accompanied the addition of fresh solution (Bohr effect) while pCO_2 remained low. In contrast, with the same rate of mechanical ventilation, the preparations perfused with canine blood were less alkalinic and the addition of fresh blood resulted in smaller variations of blood gases and *p*H parameters.

Examination of the tissue specimens after the end of the perfusion showed the lungs of the control preparations to be oedematous and in some cases haemorrhagic, while oedema and haemorrhage were absent in the haemoglobin-perfused preparations. In addition, subendocardiac haemorrhages were larger and more frequent in the blood-perfused hearts.

The maintenance of myocardial contractility and normal arterial pressure suggests that these preparations retain their ability to sustain the circulation of the intact animal. Preparations similarly preserved in another series of experiments have been transplanted successfully into suitable recipients and have supported their circulatory load for periods averaging 6 h[5,6].

That the solution adequately oxygenates the perfused tissues is further demonstrated by the high level of oxygen saturation at which it remains during the course of these experiments: a finding of particular interest, especially when compared with the behaviour of the whole blood in the control perfusions. The sharp fluctuations in *p*H and pO_2 observed in the haemoglobin-perfused preparations probably relate to the continued metabolism of the heart and lungs. In the absence of kidneys, this leads to severe alkalosis, because the haemoglobin solution has distinctly less buffering capacity than whole blood.

Oedema formation has marred the field of organ perfusion ever since its inception and has been familiar to early[7] as well as to later workers[8]. Increasing vascular resistance, decreasing flow rate, and progressive swelling have been seen as a reflexion of injury to the endothelial lining caused by small corpuscular elements. Addition of a glass wool microfilter in a heart perfusion circuit using whole blood has increased "survival" times[9]. A non-dialysable toxin or unrecognized metabolite has, however, also been implicated[10]. Protection of the capillary endothelium was therefore sought in various ways and organ preparations perfused with platelet-rich plasma[11] and the use of adjuvants such as dexamethasone[12] have yielded notable results.

These considerations certainly suggest lines for further research but do not allow more than a tentative conclusion. The pathological changes observed in our blood-perfused preparations may be caused by stromal elements of the blood damaging to endothelial walls, whereas the less severe changes seen in the haemoglobin-perfused preparations could be secondary to loss of cell membrane integrity as caused by increased metabolic toxins or corticol depletion. In any case, isolated canine heart-lung preparations perfused with a pure haemoglobin solution represent a stable system the pharmacokinetic potentialities of which seem worthy of investigation.

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Anaerobic, Glycogen-dependent Transport of Amino Acids by the Placenta

THE concentration of amino acids is greater in foetal than in maternal blood and their placental transport is fairly well established to depend on energy dependent processes[1]. Although it is reported that placental transport of amino acids occurs only in aerobic conditions[2-4], we show here that the human placenta can accumulate amino acids anaerobically against a concentration gradient, and that this is sustained by the utilization of glycogen.

Accumulation of α-aminoisobutyric acid (AIB), a non-metabolizable amino acid, was measured in slices of near-term human placental tissue in control conditions and in the presence of metabolic inhibitors. Placental slices, about 0.3 mm thick and weighing a total of 300 mg, were placed in 50 ml vessels containing 10 ml balanced Krebs saline, *p*H 7.3. After a period of preincubation designed to produce a steady state of tissue electrolytes, the slices were transferred to another flask containing buffered saline plus 0.1 mM ^{14}C-AIB, with or without 0.1 mM dinitrophenal (DNP) or 0.1 M sodium arsenate and gassed with 100% O_2 or N_2. Preincubation and incubation were carried out at the same temperature, either 25° or 37° C. After varying times the slices were removed, blotted, weighed and homogenized in 4 ml of cold 10% trichloroacetic acid. After centrifugation, aliquots of the supernatant were counted in a liquid scintillation counter. Calculations of the intracellular to extracellular accumulation ratio ($[AIB]_i/[AIB]_o$) and uptake rate (V) at 1 h in μmol per (ml intracellular water × h) were made after correcting for extracellular and total water spaces (see, for example, ref. 4). The ratio $[AIB]_i/[AIB]_o$ expresses the degree of intracellular accumulation. Values greater than 1.0 indicate transport against a concentration gradient. Placental glycogen concentration was determined using a modification of Montgomery's method[5].

Results of placental AIB accumulation at 25° C after 45 min preincubation and as a function of time in control conditions, with anaerobic atmosphere (100% N_2), DNP or arsenate, are shown in Fig. 1. Control AIB accumulation was almost linear during the first 3 h of the experiment. In the presence of N_2 or DNP, an inhibitor of electron transport in mitochondria, accumulation was decreased about 20 and 40%, respectively, but was still significantly greater than 1. (DNP concentrations of 0.05 to 0.2 mM inhibited the system to essentially the same extent indicating that 0.1 mM DNP was maximally inhibiting.) In contrast, inclusion of arsenate, an inhibitor of glycolysis, and thus of anaerobic metabolism, resulted in an accumulation ratio of about 1, a value indicating diffusion equilibrium. It should be noted that optimal AIB accumulation in anaerobic conditions depended on minimal time delay between delivery and the start of the experiment. AIB uptake rates in μmol/(ml × h) were: control=0.18 (±0.06); N_2=0.14 (±0.03); DNP=0.10 (±0.02).

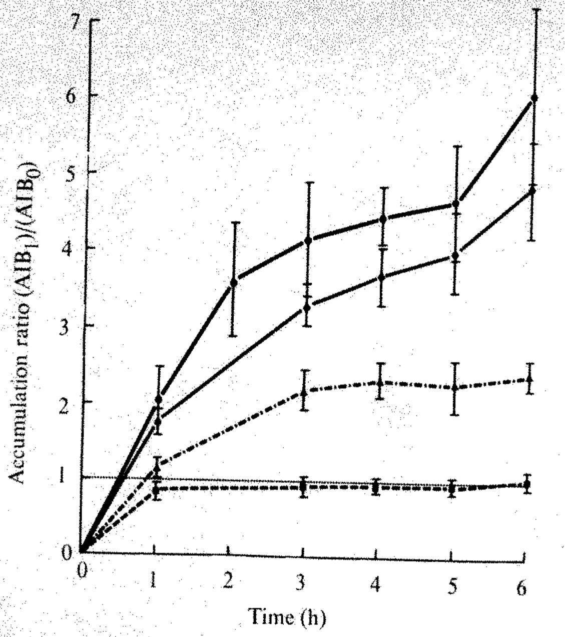

Fig. 1 Ratio of concentration of α-aminoisobutyric acid (AIB) in free water of placental tissue to its concentration in the media, as a function of time at 25° C following 45 min preincubation. Control (●); 100% N_2 atmosphere (◆); 10^{-4} M dinitrophenol (▲); 10^{-1} M sodium arsenate (■). An accumulation ratio of 1 indicates diffusion equilibrium. Each point is the mean of nine determinations on these animals; bars represent 1 s.d.

That glycogen is the energy source for the accumulation is suggested by an increased rate of disappearance of this storage polysaccharide in anaerobic conditions. Placental glycogen concentration was about 27% lower than control values both 1 and 2 h after incubation of the slices simultaneously in N_2, DNP and 0.055 mM adrenaline.

If the tissue slices were preincubated at 37° C for 3 h under N_2 and in the presence of DNP and epinephrine, so as to remove or considerably reduce available glycogen, anaerobic AIB accumulation was nearly abolished (Fig. 2). Control accumulation ratios were considerably higher after a 3 h preincubation than after a 45 min preincubation (compare Figs. 1 and 2). This phenomenon, which is not understood at present, seems to be only partly due to the higher temperature and is under investigation.

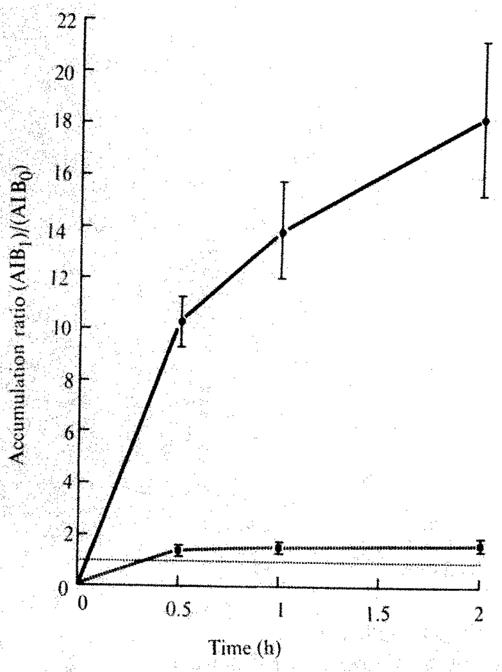

Fig. 2 Accumulation ratio for AIB at 37° C after a 3 h preincubation without (●) and with (■) simultaneous N_2 atmosphere, 10^{-4} M DNP and 5.5×10^{-5} M adrenaline to decrease available energy source. Each point is the mean of nine determinations on these animals; bars represent 1 s.d.

That the lower accumulation ratio is truly due to the removal of the energy source and not to cell death is shown by the addition of glucose to the tissues after anaerobic preincubation to reduce available glycogen (Fig. 3). In the presence of 10 mM glucose, the slices can once again accumulate amino acids anaerobically (Fig. 3), whereas accumulation was minimal in the absence of glucose.

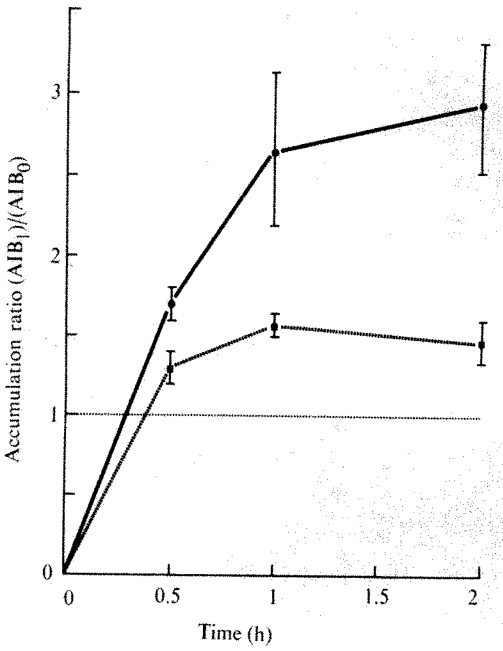

Fig. 3 Accumulation ratio of AIB at 37° C after 3 h preincubation with simultaneous N_2 atmosphere, 10^{-4} M DNP and 5.5×10^{-5} M adrenaline. In the presence of added 10 mM glucose (●) the accumulation ratios were significantly greater than when glucose was not added (■). Each point is the mean of nine determinations on three animals; bars represent 1 s.d.

Sybulski and Tremblay[2] reported that N_2 and 1.3 mM DNP decreased glycine uptake by human placental slices about 27% and 38% respectively. Whether or not accumulation against a concentration gradient occurred cannot be determined from their data. Litonjua *et al.*[3] also reported decreased AIB uptake by human placental slices under 100% N_2 with no accumulation noted. Dancis *et al.*[4] reported that 0.2 mM DNP and 10 mM sodium cyanide decreased $[AIB]_i/[AIB]_o$ about 34% and 62% respectively in 1 h from a control value of about 2.25. The actual values were not given and presumably the accumulation ratios were 1 or less.

Compatible with our results is the finding of Reynolds and Young[6] that the rate of α-amino acid nitrogen transfer in the *in situ* perfused guinea-pig placenta was not decreased when maternal arterial O_2 tension was lowered to 30 mm Hg, or by the addition of either 0.5–5 mM DNP or 2–10 mM KCN to the perfusate. Whether active accumulation was affected by these procedures was not noted.

Although all tissues possess the glycolytic pathway and therefore have the potential for supporting energy-dependent processes by anaerobic metabolism, anaerobic amino acid transport does not occur in adult intestine[7–9] or kidney[10].

On the other hand, foetal tissues have an unusual propensity to function independently of oxidative phosphorylation compared with adult tissues[11]. Foetal and newborn rabbit small intestine anaerobically transports L-histidine, L-tyrosine and 6-deoxy-D-glucose[8]. Butt and Wilson[9] showed that embryonic guinea-pig yolk sac and intestine transport amino acids anaerobically. Whittam[12] reported anaerobic maintenance of Na^+, K^+ and water concentrations in immature and newborn rabbit and rat kidney cortex slices. Near term foetal liver slices have been shown[13] to carry out lipogenesis anaerobically more rapidly than adult liver. Term placental slices continue to utilize fructose and glucose after 1 h of complete anoxia[14].

Our studies suggest that glycogenolysis can support placental transport in anaerobic conditions. Supporting this, Demers *et al.*[15] reported that DNP and severe hypoxia decreased the glycogen content of placental villi in organ culture with a shift from glucose-6-phosphate-dependent to independent glycogen synthetase (the enzyme catalysing glycogen synthesis), and a concurrent activation of glycogen phosphorylase (a glycogen-degrading enzyme).

To what extent anaerobic transport serves as an adaptation to increase foetal survival during periods of acute hypoxia cannot be stated. It would seem that the foetal brain would suffer irreversible damage before the foetus suffered from amino acid deficiency. Perhaps the ability to function anaerobically has survival value for the foetus at an early period in gestation; for example, before development of the central nervous system is complete. In support of this, glycogen utilization by human placenta occurs at a substantially greater rate during the first third of gestation than thereafter[16].

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Note added in proof. Smith *et al.*[17] have also shown recently that the enhancement of placental AIB accumulation is a function of prolonged pre-incubation.

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Complementation Analysis of Maple Syrup Urine Disease in Heterokaryons derived from Cultured Human Fibroblasts

Maple syrup urine disease (MSUD) is an autosomal recessive disorder characterized by keto acidosis, convulsions, mental retardation and a maple syrup odour in the urine. The clinical phenotype varies among different families, with a severe early onset in the classical form of the disease and milder symptoms with delayed onset in variants. The biochemical defect is an inability to degrade the three branched-chain amino-acids (BCAA), leucine, isoleucine, and valine, as a result of a deficiency in decarboxylation of the branched-chain α-keto acids (BCKA). The enzyme defect is expressed in skin fibroblast cultures derived from affected patients, and the level of residual BCKA decarboxylase activity correlates with the severity of clinical symptoms. The heterogeneity in clinical presentation and the level of residual decarboxylase activity appear to be genetically determined[1].

Intergenic[2,3] and possibly interallelic[4,5] complementation of other mutations has been reported in fused mammalian cells. We approached genetic heterogeneity in MSUD by using Sendai virus-induced fusions of skin fibroblasts derived from unrelated patients. If fusing two different mutant cells corrects their common BCKA decarboxylase deficiency it is assumed that they have different genetic lesions. In MSUD the efficiency of complementation is high in certain heterokaryons and can be detected without a selective system. The ability of MSUD fibroblasts to complement one another bears no apparent relationship to the severity of the disease or the level of residual BCKA decarboxylase activity.

Fibroblast strains derived from skin biopsies were grown in Waymouth's medium containing 15% foetal calf serum and antibiotics[6]. The severity of MSUD in the patients from whom fibroblasts were derived was classified on the basis of clinical phenotype, dietary tolerance for BCAA, and residual BCKA decarboxylase activity in fibroblast cultures[1]: BaKu and TeHe—grade 1: classical MSUD (0–2% of normal enzyme activity). NiRo—grade 2: variant MSUD (2–8% of normal enzyme activity). ElHa—grade 3: variant MSUD (8–15% of normal enzyme activity).

BCKA decarboxylase was assayed in intact cell suspensions rather than cell lysates because BCKA decarboxylase activity is greatly reduced in disrupted normal or mutant fibroblasts. The branched-chain amino-acids, labelled in the C-1 position, were used as substrates because lower and more consistent blanks were obtained with them than with the branched-chain α-keto acids. The first degradative step, transamination, is not rate limiting in normal or MSUD subjects[7]. BCKA decarboxylase activity of the cells is expressed as the ratio of radioactive CO_2 evolved to the amount of radioactivity incorporated into protein (TCA precipitated material), the latter serving as a measure of the metabolically active cells[1,7]. Within each experiment the total number of cells per flask, total cell protein per flask, and the incorporation of radioactive BCAA into TCA precipitated material was similar for replicate cell mixtures with and without added Sendai virus. Increased ratios of CO_2 to protein, such as those observed in complementing cell pairs treated with virus, were due to increased evolution of radioactive CO_2 rather than selective reduction in protein synthesis.

The method of Velasquez *et al.*[8] yielded the greatest number of heterokaryons and the best recovery of viable cells. The number of nuclei per cell varied from two to as many as six or seven, but most of the fused cells had either two or three nuclei. For complementation studies the hybridized cells were maintained in the original confluent monolayers, thus few cell divisions occurred after cell fusion. Nuclear fusion was not observed in our preparations.

Table 1 shows the result of studying confluent cultures of fibroblasts grown from single individuals. Multinucleated cells were 15–30% of the total cell number in virus treated flasks, but the BCKA decarboxylase activity of these flasks was not significantly different from that of untreated flasks whether the cell strains were derived from normal subjects or from patients with classical or variant forms of MSUD (complementation ratio = 1). Similarly, if fusion was induced between fibroblasts derived from siblings with MSUD, who presumably have the same genetic lesion, there was no increase in enzyme activity (not shown). The two experiments carried out with GaSa (normal) in Table 1 show that the BCKA decarboxylase activity of a cell strain can vary from experiment to experiment. The method of assaying intact cells, involving complex handling of small numbers of cells which may differ in metabolic efficiency, probably introduces this variability. Within each experiment, however, we can obtain consistent measurements of enzyme activity in replicate flasks, thus allowing comparisons between virus-treated and untreated (control) flasks which have been handled similarly.

Once it was clear that the events of fusion, including the addition of virus, did not affect BCKA decarboxylase activity, fusion between MSUD strains were undertaken. BaKu has the clinical symptoms of classical MSUD, but when fibroblasts from this patient were fused with those of TeHe, NiRo, and ElHa, the virus-treated flasks consistently had BCKA decarboxylase activities at least three times greater than those of the same cell mixtures which were not treated with virus (Table 2). In each instance the enhanced activity was the result of increased evolution of radioactive CO_2 while the radioactive TCA-precipitated material remained constant. Complementation occurred regardless of whether the partners of BaKu had similar or different clinical manifestations of MSUD.

Table 1 Effect of Inactivated Sendai Virus on BCKA Decarboxylase Activity in Homokaryons

		BCKA decarboxylase activity *				
Cell line	Diagnosis	Control cells		Fused cells		Complementation ratio †
		Individual flasks	(Average)	Individual flasks	(Average)	
MuJa	Normal	179.4		145.7		
		150.2	(164.8)	170.5	(187.2)	1.1
				245.5		
GaSa	Normal	158.5		148.5		
	(Exp. 1)	163.8	(170.6)	172.1	(167.0)	1.0
		189.4		180.3		
	(Exp. 2)	404.5		414.2		
		396.7	(395.5)	370.4	(386.4)	1.0
		385.2		374.7		
BaKu	Classical	0		0		
		0	(0)	0	(0)	(1)
		0		0		
TeHe	Classical	0		0		
		0	(0)	0	(0)	(1)
		0		0		
ElHa	Variant	15.8		15.4		
		21.2	(20.9)	19.4	(18.6)	0.9
		25.8		20.9		

Cells were collected from monolayer cultures, suspended in Waymouth's medium containing 10% immunoprecipitation tested (IPT) foetal calf serum, and inoculated into replicate T-30 flasks (Falcon) at concentrations of 10^5 cells cm^{-2} of surface area (2.5×10^6 cells per flask). They settled into dense monolayers within 6–8 h at 37° C. Sendai virus, inactivated with β-propriolactone[12], was added to half of the flasks at a concentration of 1,000 to 5,000 haemagglutination units per flask. The flasks without virus served as controls. After overnight incubation, the medium was decanted, fresh medium containing 10% foetal calf serum was added, and the flasks were maintained with routine feeding until assay from three to eight days later. The virus-treated flasks contained 15–30% multi-nucleated cells while control flasks had 1–2%.

BCKA decarboxylase was measured by collecting the cells from replicate flasks with a brief exposure (1–2 min) to trypsin (0.04%) and versene (0.02%) in Puck's saline A, followed by scraping with a rubber policeman. Each suspension was diluted with foetal calf serum so that the final serum concentration was 20%, centrifuged at 800 r.p.m. for 5 min, and washed once with Hank's balanced salt solution. After a second centrifugation, the cell pellets were resuspended in 0.28 ml. of Krebs-Ringer phosphate buffer (*p*H 7.1–7.3) containing 1.75 mg $ml.^{-1}$ thiamine HCl and added to 21×70 mm homoeopathic vials which contained 0.5 μmol (0.2 μCi) of [1-^{14}C]-L-leucine. Less than 1% of the substrate was metabolized in the assay. Volatile radioactive contaminants were removed from the substrate before incubation as previously described[1,7]. The vials were stoppered with serum vial caps carrying centre wells (Kontes, K882310 and K882320) containing 0.1 ml. of 1 N KOH, flushed with oxygen, and incubated at 35° C for 90 min with gentle swirling in a New Brunswick shaker water bath. The evolution of CO_2 is proportional to time for at least 120 min.

The reaction was ended by injecting 0.3 ml. of 10% TCA into the medium through the stopper, and the incubation was continued for an additional 60 min to complete the collection of dissolved CO_2. The contents of the wells were transferred quantitatively to scintillation vials containing 15 ml. of scintillation cocktail[1]. Reagent blanks normally liberated 20–40 c.p.m. of $^{14}CO_2$.

The TCA-precipitated cells were collected by adding 5 ml. of 5% TCA to the reaction vials and transferring the contents to centrifuge tubes. The precipitates were washed once with additional 5% TCA, once with distilled water and dissolved in 0.2 ml. of 1 N NaOH at 60° C for 2–4 h. The digests were added to scintillation cocktail to determine radioactivity.

BCKA decarboxylase activities in cultures fused with Sendai virus were compared to untreated replicate cultures (controls) prepared from the same cell suspension. Within an experiment the conditions of culture, the preparation of cells for assay and the measurements of BCKA decarboxylase activities were the same for the virus-treated and control cells.

* Ratio of $^{14}CO_2$ per 100 c.p.m. in TCA-insoluble material.

† Complementation ratio is the average BCKA decarboxylase activity of fused cells divided by that of control cells.

Table 2 Fusions between BaKu and other MSUD Fibroblast Strains

			BCKA decarboxylase activity				
BaKu (Classical) Fused with:	Expt.	Day of assay	Control cells		Fused cells		Complementation ratio
			Individual flasks	(Average)	Individual flasks	(Average)	
TeHe	1	4	10.8		38.8		
(Classical)			0	(3.6)	18.0	(22.7)	6.3
			0		11.3		
		5	2.1		15.5		
			0	(0.7)	31.7	(21.5)	30.7
			0		17.3		
		6	2.3		31.2		
			5.3	(4.7)	36.0	(35.1)	7.5
			6.5		38.2		
	2	3	6.0		23.1		
			4.0	(8.0)	38.2	(32.0)	4.0
			14.0		34.6		
		4	5.8		27.9		
			6.7	(10.0)	46.6	(41.2)	4.1
			17.5		49.0		
NiRo	3	5	41.7		138.5		
(Variant)			31.1	(37.8)	141.4	(134.2)	3.6
			40.5		122.7		
	4	4	7.5		32.4		4.3
	5	3	10.9		52.9		
			8.0	(9.3)	41.0	(43.7)	4.7
			9.1		37.8		
ElHa	6 *	6	21.2		70.6		
(Variant)			19.9	(20.6)	55.8	(63.2)	3.1
	7	4	23.0		84.5		
			14.2	(17.6)	85.6	(75.7)	4.3
			15.7		56.9		
		6	11.6		61.0		
			9.4	(11.1)	46.6	(54.3)	4.9
			12.2		55.2		

Fibroblasts from two individuals were mixed in equal numbers and distributed among T-30 flasks. Half the flasks were treated with inactivated Sendai virus and half were controls. The BCKA decarboxylase activities were determined with leucine as substrate at the same time on different days after fusion.

Decarboxylase activity and complementation ratios are reported as described in Table 1.

* ElHa fibroblasts from exp. No. 6 when assayed alone had BCKA decarboxylase activity of thirty and thirty-one units.

Table 3 Fusions among MSUD Fibroblast Strains

			BCKA decarboxylase activity				
			Control cells		Fused cells		
Fusion	Expt.	Day of assay	Individual flasks	(Average)	Individual flasks	(Average)	Complementation ratio
TeHe × ElHa (Classical × variant)	1	4	9.9		17.9		
			13.5	(20.1)	4.8	(15.8)	0.8
			36.8		24.6		
	2	7	49.6		42.8		
			50.3	(52.8)	44.8	(49.2)	0.9
			58.6		60.1		
NiRo × ElHa (Variant × variant)	3	4	76.5		29.5		
			89.9	(88.4)	25.5	(34.3)	0.4
			98.9		48.0		
		6	79.3		41.4		
			88.3	(83.8)	31.3	(35.0)	0.4
			83.7		32.3		

See legends of Tables 1 and 2.

The effectiveness of BaKu in complementing the three other mutants suggests that this patient's genetic lesion is distinct from the others, but it does not necessarily imply that the other three have the same genetic lesion. This aspect was investigated by fusions among these three (Table 3). BCKA decarboxylase activity did not change significantly when TeHe was fused with ElHa. Similar studies with NiRo and ElHa, however, produced a distinct depression of BCKA decarboxylase activity. This might suggest that the mutations of these two subjects differ such that their interaction lowers the effectiveness of the enzyme, perhaps by a mechanism similar to that of complementation but with a negative effect. Clinically, the possibility arises that the progeny of two such patients might be more enzyme deficient than either parent.

The genetic lesion in MSUD causes approximately parallel deficiencies in decarboxylase activity toward the three branched-chain amino-acids, suggesting that in normal individuals a single enzyme is responsible for decarboxylating all of them. When leucine and valine were compared as substrates for the activity produced by complementation restoration of decarboxylase activity for leucine (complementation ratio 3.9) was much greater than for valine (ratio 1.5) in a typical experiment. Although this finding may indicate that there is a separate decarboxylase enzyme for each substrate, it might also be the case that the enzyme activity restored by complementation differs enough from the wild-type to metabolize the substrates selectively. Interallelic complementation in microbial systems and in eukaryotic cells often restores enzyme activity with kinetic properties different from the wild-type.

The enhanced BCKA decarboxylase activity found in virus-treated mixtures of cells from certain patients deficient in this enzyme activity strongly suggests that complementation has occurred in heterokaryons produced by cell fusion. Complementation appears to be selective, as it was observed with some pairs of cell strains but not with others. This finding indicates that MSUD fibroblast strains fall into at least two complementation groups which do not appear directly to be related to the clinical expression of the disease. An important finding in our experiments is that this technique can be used to study monolayer cultures which have been exposed to inactivated Sendai virus without further selection. The complementation that we observe must be very efficient, as we estimate that only about 18% of the virus-treated cells were actual heterokaryons, based on random fusion of the two cell types. Thus the level of BCKA decarboxylase activity may be close to the normal level in the few heterokaryotic cells which can express the enhancement.

It is not possible, on the basis of our observations, to distinguish between intergenic and interallelic complementation. The peptide composition of the BCKA decarboxylase and the number of genetic loci which contribute to the enzyme complex are unknown. Complementation occurs in conditions of culture where nuclear fusion is unlikely. This strongly suggests that in our heterokaryons cytoplasmic interactions are responsible for complementation. BCKA decarboxylase seems to have a mitochondrial localization in liver[9-11] and to be part of a large multicomponent enzyme complex. To determine how complementation occurs requires a cell-free enzyme preparation, but we have been unable to recover significant BCKA decarboxylase activity from disrupted normal or mutant fibroblasts.

Complementation analysis provides a potentially important method for studying the genetic heterogeneity observed in many human biochemical disorders. For example, complementation can reveal functional differences between mutations of independent origin. Using these techniques it may be possible to construct complementation maps of various inborn errors and eventually to understand the molecular basis of the disease at the level of the deficient enzyme.

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Role of Inositol Cyclic Phosphate in Stimulated Tissues

THYROID-STIMULATING hormone markedly stimulates phosphatidylinositol turnover in sheep thyroid gland. The enzyme catalysing the degradation of phosphatidylinositol in animal tissues can split the phospholipid, with the formation of a diglyceride and a new cyclic ester, D-*myo*inositol-1:2-cyclic phosphate as well as D-*myo*inositol-1-phosphate[2,3]. Increased phosphatidylinositol turnover has been described in a variety of conditions in which cells are stimulated to secrete or into other hyperactivity. The physiological possibilities of this degradation are obvious, and it was suggested[2,3] that the catabolism of phosphatidylinositol and the release of inositol cyclic phosphate might be more directly relevant to the cell stimulation than to the biosynthesis involved in the turnover. Such an hypothesis is attractive, for an accumulation of phosphatidylinositol has never been demonstrated during stimulated conditions. In addition Ca^{2+} is an essential requirement for the formation of inositol cyclic phosphate as well as playing an important part in secretory processes[4].

The demonstration of a specific phosphodiesterase in many mammalian tissues which hydrolyses only the D-enantiomorph of inositol cyclic phosphate, producing inositol-1-phosphate[5], also suggested a defined function for the metabolite. We therefore tested whether a direct role for inositol cyclic phosphate could be demonstrated in the phenomena associated with increased phosphatidylinositol turnover of stimulated tissues. Michell and Lapetina[6,7] have expressed similar speculative views concerning the possible role of inositol cyclic phosphate in the stimulated turnover of phosphatidylinositol.

The phosphodiesterase which attacks inositol cyclic phosphate is similar to the enzyme hydrolysing adenosine cyclic phosphate, having a requirement for Mg^{2+} and an alkaline *p*H optimum[5]. But we have found that the enzymes have distinct identities. Thus, the distribution of inositol-1:2-cyclic phosphate-2-phosphohydrolase is quite different from that of cyclic AMP phosphodiesterase in the rat kidney nephron. The former enzyme is confined to the brush borders of the pars convoluta in the outer kidney cortex, whereas the latter occurs chiefly in a soluble fraction obtained from the medulla and papilla. Furthermore, kidney inositol-1:2-cyclic phosphate-2-phosphohydrolase is insensitive to theophylline, a potent inhibitor of cyclic AMP phosphodiesterase, in the same tissue[8,9]. We have also shown, in collaboration with Drs L. Birnbaumer and E. Whitmore (Department of Physiology, Northwestern University Medical School) that inositol cyclic phosphate does not act as a competitive inhibitor of cyclic AMP phosphodiesterase. These experiments were performed with a variety of rat tissues using a substrate concentration of 0.25 μM ^{32}P-labelled cyclic AMP (high affinity enzyme)[10]: even inositol cyclic phosphate at a concentration of 10^{-3} M produced no inhibition.

In additional experiments performed in collaboration with Dr L. Birnbaumer we found that inositol cyclic phosphate at a final concentration of 10^{-4} M had no effect on the adenylcyclase system of renal medullary membranes. This activity was determined at two [α^{32}P] ATP concentrations (5 and 64 μM) and in the absence and presence of arginie, vasopressin, prostaglandin E_1, GTP and adenosine—agents which profoundly affect the activity of the cyclase.

In further experiments in collaboration with Dr J. F. Wilber (Department of Medicine, Northwestern University Medical School) we tested whether inositol cyclic phosphate had any effect on the elaboration of growth hormone (GH) and thyroid stimulating hormone (TSH) by hemisected rat anterior pituitaries. The pituitary shows an increased turnover of phosphatidylinositol in response to secretory stimulation[11]. The excised rat hemipituitaries, after a stabilizing incubation, were incubated with and without inositol cyclic phosphate and the release of growth hormone and thyroid stimulating hormone determined by radio-immunoassay[12]. No enhanced TSH or GH release was seen with pure Na inositol cyclic phosphate at concentrations up to 10 mM.

Increased phosphatidylinositol turnover is one of the primary events when lymphocytes are induced to transform with phaetohaemagglutinin[13] or concanavalin A (unpublished results of M. Smith and R. M. C. D.). In collaboration with Dr A. C. Allinson (National Institute for Medical Research, Mill Hill, London), however, we found no evidence that inositol cyclic phosphate could by itself transform sheep lymphocytes. Nor did concanavalin A increase the ^{32}P labelling from ^{32}P-inorganic phosphate of a fraction from lymphocytes containing inositol cyclic phosphate, even though the labelling of phosphatidylinositol was increased ten-fold in the first hour. In addition, in experiments with Dr G. E. Shambaugh III (Department of Medicine, Northwestern University Medical School) in which human lymphocytes were prelabelled by incubation with ^{14}C-inositol, we could not demonstrate a measurable release of labelled inositol cyclic phosphate within the lymphocytes when phaetohaemagglutinin was added to the incubation medium.

Finally, we prelabelled isolated pancreatic islets of the rat by incubation with both ^{32}P-phosphate and ^{3}H-inositol. On perfusion of such islets according to the technique of Lacy *et al.*[14] an increase of glucose concentration from 50 to 300 mg/100 ml. almost immediately elicited a stimulated secretion of immunoreactive insulin. But no release of inositol cyclic phosphate or its immediate enzymatic decomposition product, inositol-1-phosphate, could be detected, either in the islets themselves or in the perfusion fluid. Stimulation of insulin release from rat pancreas slices by an elevated glucose concentration is again attended by an increased incorporation of precursor isotopes into phosphatidylinositol[15].

In none of the systems which we have examined, and within the limitations of existing analytical techniques, can we find evidence that in a stimulatory situation there is an increased level or release of the metabolite inositol cyclic phosphate. Nor can we find any evidence that this metabolite can enhance hormone release, transform lymphocytes or affect the formation or breakdown of cyclic AMP. Of course, it is almost impossible to prove a negative physiological function for any individual metabolite, particularly when tissues contain a very active enzyme system for degrading this metabolite. So far, however, we have been unable to secure evidence that the enhanced turnover of phosphatidylinositol observed during many types of tissue stimulation can be ascribed to a need to produce inositol cyclic phosphate, and further speculation on this score without firm experimental data is useless.

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Oviposition Pheromone in Larval Mandibular Glands of *Ephestia kuehniella*

WHEN larvae of *Ephestia kuehniella* Zeller meet they deposit from their mandibular glands a pheromone that mediates a response to crowding[1]. In this communication I show that ovipositing female moths of *E. kuehniella* respond to the same secretion in such a way that the number of eggs they lay is related to the amount of the larval pheromone (and so to the density of the population of larvae) at the oviposition site.

The experimental animals were kept at 25° C in a photoperiodic regime of 12 h light : 12 h dark, from the wandering stage of the last larval instar onwards. Males and females were kept in separate incubators. Each day at lights-off the moths that had emerged during the past 24 h were removed from the emergence jars. Twenty-four hours later the females were put with an equal or greater number of males of the same day-group; and 24 h after that the females (2-d-old mated females) were used in experiments. Although most of the females treated in this way mate[2], some of the females that laid few eggs contained unusually small spermatophores and some contained none. Females that laid fewer than ten eggs have therefore been omitted from the analysis.

Two-day-old mated females, placed singly in an inverted vial supported over a horizontal disk that rotated once in 24 h, laid most of their eggs on the disk just after the beginning of the dark period, sometimes showing a smaller peak of oviposition before lights-on (Fig. 1). The number of eggs decreased from a maximum on the first day after mating to a very few on the fourth. The experiments described here were arranged so that fresh mandibular gland material was presented to the 2-d-old mated female moths at lights-off, just before the first oviposition peak.

In the first experiment, 2-d-old mated females of *E. kuehniella* were put singly into plastic boxes (of internal dimensions 56×38×13 mm) in which wandering larvae of the same species had just been confined (singly or in groups of five, ten, twenty or fifty) for 1 h at 25° C. These larvae were removed from the boxes a few minutes before lights-off, when the moths were put in and allowed to oviposit for 24 h. Figure 2 shows that the number of eggs laid in each box is related to the number of larvae previously crowded there; more eggs were laid in boxes that had contained about twenty larvae than in boxes that had contained more or fewer larvae. The second experiment showed that this response could be mediated by mandibular gland secretion deposited by the crowded larvae. Mandibular glands from wandering larvae were ground up in ether, and 50 μl aliquots of various dilutions of the ether extract were put onto 16 mm diameter coverslips. Within 15 min of lights-off, 2-d-old mated female moths were put singly into standard boxes, each with a treated coverslip, and allowed to oviposit for 6–7 h. Figure 3 shows that more eggs were laid at about 0.05–0.5 pairs of glands per coverslip than were laid at higher or lower concentrations of mandibular gland extract. The concentration of the mandibular gland extract influenced not only the number of eggs laid during the first oviposition peak but also the spatial distribution of the eggs; the percentage of eggs laid on or under the coverslips was higher for those treated with 0.5 pairs of glands than for those treated with higher or lower concentrations of extract.

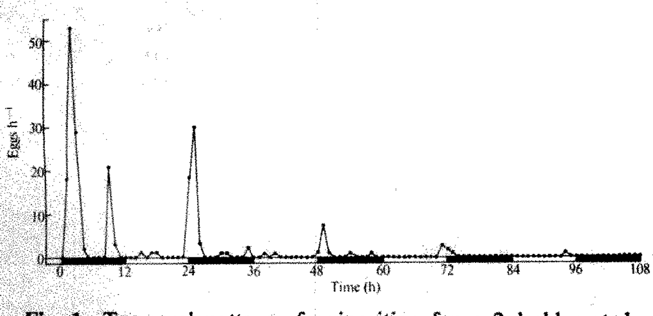

Fig. 1 Temporal pattern of oviposition for a 2-d-old mated female moth in a photoperiodic regime of 12 h light : 12 h dark (indicated on the abscissa).

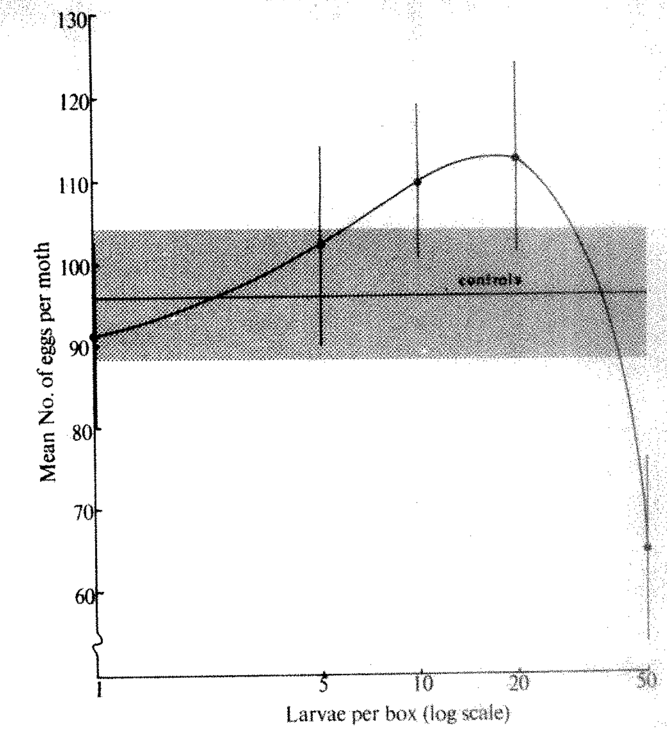

Fig. 2 Numbers of eggs laid in 24 h by 2-d-old mated female moths confined alone in standard boxes that had been previously inhabited for 1 h by single or crowded larvae. The horizontal line represents the mean number of eggs laid in control boxes that had not contained larvae. The vertical lines (and the extent of stippling for the controls) represent 1 s.e. each side of the mean.

Ether extract of mandibular glands contains several components, but it is possible to obtain one of them almost pure by squeezing the oily fluid out of mandibular glands under water and drawing off the water (compound I of Mudd and Corbet[3]). The third experiment showed that the effect on oviposition of material prepared in this way was similar to that of the ether extract. Within 15 min of lights-off, twenty-one 2-d-old mated female moths were put singly into standard boxes, each of which contained a 16 mm diameter coverslip bearing the squeezed-out contents of one mandibular gland from a wandering larva. This oily droplet was dispersed over the coverslip by adding a 50-μl aliquot of ether. In the first 6 h from lights-off these moths laid an average of 123 eggs each (s.e.=14). A parallel series of twenty moths with clean coverslips laid significantly fewer eggs (mean egg number=97; s.e.=9; $P<0.05$, one-tailed Mann-Whitney U test). Further, the proportion of

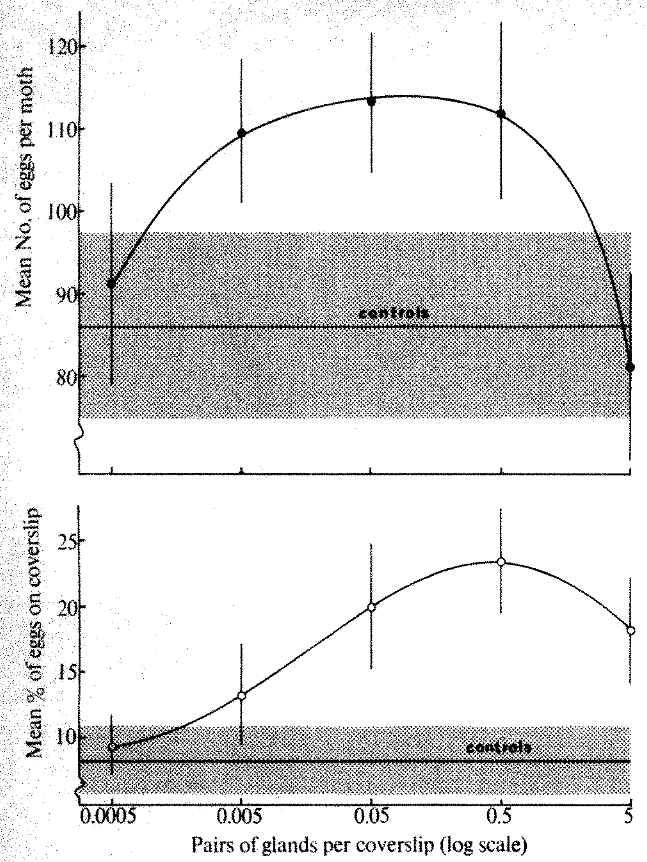

Fig. 3 Influence of concentration of ether extract of mandibular glands on (*a*) the number of eggs laid in 6–7 h by 2-d-old mated female moths; and (*b*) the percentage of those eggs that were laid on the coverslip bearing the extract. The horizontal lines represent the mean values for controls with untreated coverslips. The vertical lines (and the extent of stippling for the controls) represent 1 s.e. each side of the mean.

eggs laid in contact with the coverslip was significantly higher for the treated coverslips (mean=22%; s.e.=4%) than for the clean ones (mean=9%; s.e.=1%; $P<0.01$, one-tailed Mann-Whitney U test).

The response of larvae to larval mandibular gland secretion could contribute to the regulation of population density, affecting the dispersion of larvae, the duration of larval development and the size (and therefore the fecundity) of moths crowded as larvae[1]. The experiments described here show that the moths, too, respond to the secretion in a way that could contribute to the regulation of population density. The larval and the imaginal responses show similar patterns of change with the concentration of mandibular gland extract. The duration of the last larval instar is least at an intermediate concentration of gland material (corresponding to the material deposited by five larvae in a standard box in 1 h) and longer at lower or higher concentrations; and the number of eggs laid by moths is greatest at an intermediate concentration (corresponding to the material deposited by about twenty larvae in a standard box in 1 h) and less at higher or lower concentrations. The pattern of the moths' response may enable them to select an oviposition site that is capable of supporting larval growth, but is not over-populated.

Among pheromones with potential for the control of insect pests those affecting the time or place of oviposition are especially worth attention as they can be used to prevent or reduce infestation by repelling gravid females or by luring them into traps. Compound I, which is probably at least partly responsible for the effects on oviposition described here, has been identified in the larval mandibular glands of three other phycitid pests of stored products, *Ephestia cautella* (Walk.), *E. elutella* (Hübn.) and *Plodia interpunctella* (Hübn.) as well as *E. kuehniella*[3], and work is in progress to establish its structure.

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Ventilation of Toad Lungs in the Absence of the Buccopharyngeal Pump

T. H. HUXLEY[1] stated "Pulmonated Vertebrata, which have the thoracic skeleton incomplete (as the Amphibia) breathe by distending their pharyngeal cavity with air; and then, the mouth and nostrils being shut, pumping it, by the elevation of the hyoidean apparatus and floor of the pharynx, into the lungs. A frog therefore cannot breathe properly if its mouth is kept wide open". This account of amphibian respiration and the buccopharyngeal "pump" has been perpetuated in biological texts (for instance, refs. 2–7) but, as our observations on the toad *Bufo marinus* indicate, may not be completely correct.

The mouths of the toads (*Bufo marinus*, 150 to 350 g) were held open by thrusting a piece of plastic tubing between the jaws. These toads cannot inflate their lungs to the balloon-like proportions often seen, the buccopharyngeal pump being necessary for this and also undoubtedly for normal respiration. These toads, however, rapidly (20–30 per min; mean 26 for 7 toads) oscillated their dorsal thoracic wall. Oxygen consumption was greatly in excess of cutaneous oxygen uptake[8–10], thus confirming that ventilation of the lungs did occur.

Table 1 Oxygen Consumption of *Bufo marinus* (at 22° C). Effects of Preventing Ventilating Action of the Buccopharyngeal Pump

	Oxygen consumption ml. kg^{-1} h^{-1}		
Wt. of toad (g)	Normal "resting"	Buccopharyngeal "pump" blocked (tube in mouth)	Injected with neuromuscular blocker (gallamine)
145	41	84	—
351	42	113	42
311	44	150	35
265	57	252	55
261	43	166	55
240	63	106	46
237	33	148	54
227	64	179	46
Mean ± s.e.	48 ± 4.0	150 ± 18.5	48 ± 2.9

The rate of resting oxygen consumption (measured with a paramagnetic oxygen analyser) is shown in Table 1. When the tube was placed between the jaws oxygen consumption did not decline, as may be expected if ventilation of the lungs ceased, but rose considerably. This continued for at least 2 h. The increased metabolism may reflect excitement as well as increased muscular activity of breathing. The toads did not appear distressed and the breathing movements were not gasping ones. To prevent movements of the thoracic muscles and thus stop ventilation of the lungs, we injected a neuromuscular blocking drug (gallamine triethiodide, 40 mg per 150–350 g toad). After 60 to 90 min the thoracic breathing movements usually ceased. They retained the eye blinking reflex, however, and buccopharyngeal movements could still occur so that the mouth-tube was left in place. In these toads, oxygen consumption declined to the original "resting" value. This presumably reflects enhanced cutaneous oxygen uptake.

There has been considerable speculation regarding the possible respiratory mechanism present in early amphibians. Cox[11] considers that the ancestral Lissamiphibian relied solely on hyoid (positive-pressure pumping) ventilation supplemented by cutaneous gas exchange. Gans[12], on the other hand, has suggested that such amphibians must have utilized aspiratory (negative-pressure type) ventilation and that the hyoid ventilation in the frog is a "red herring". The present observations indicate that negative-pressure type ventilation may occur even among contemporary amphibians despite the lack of a diaphragm and rib cage. Thus the present experimental observations pose two fundamentally interesting questions. First, how essential is the rib cage and diaphragm for negative-pressure type "ventilation"? Second, need we assume that the earliest terrestrial vertebrates possessed a buccopharyngeal pump?

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Absence of Y-Body in the Cervical Mucus of Pregnant Women

SHETTLES[1] reported the possible use of the fluorescent Y-chromatin test[2] for prenatal sex diagnosis in smears of maternal cervical mucus. This technique, if it works, could be very valuable, particularly during the first trimester of pregnancy when amniocentesis is impracticable. We have tested the feasibility of the technique in sixteen pregnant women who were admitted for legal abortion in the first trimester, that is, from the seventh to twelfth week of gestation after the first day of the last menstrual period.

Smears obtained from mid-cervical mucus with a cotton swab were fixed in 3:1 methanol–acetic acid for 1–2 min, air-dried and stained with quinacrine mustard (50 μg ml.$^{-1}$ in MacIlvaine citric acid-phosphate buffer, pH 4.5) for 10 min. They were washed in running tap water, rinsed three times and mounted in the same buffer. In each sample 100–800 interphase nuclei were scanned for the Y-body, using a fluorescence microscope. Conceptuses were then taken out by curettage, and fragments of the embryo and chorionic villi separated, washed in saline, minced with scissors, and smeared on slides. The slides were examined for the Y-body as described above. The X-chromatin of both villi and embryonic tissues was examined according to the acetic orcein squash method, on the basis of 100 nuclei in each sample. In addition, a portion of the embryonic tissue was cultured *in vitro* for direct analysis of sex chromosomes.

Twelve of the sixteen embryonic cell cultures showed a 46,XY karyotype and the remaining four a 46,XX karyotype. The X-chromatin findings in both chorionic villi and embryonic tissues conformed with the results of the karyotype analysis. In spite of the relatively high proportion of male conceptuses involved, we failed to detect typical Y-body in any of the sixteen samples of cervical smears, which were scanned blind on the basis of a total of 4,000 well-fixed nuclei. In striking contrast, the smears of chorionic villi and embryonic cells from the twelve chromosomally verified male specimens, showed a very high incidence of the Y-body; it was present in 70% of villi smears and 92% of embryo smears. Smears from the remaining four cases did not show Y-body in the villi, embryonic cells and the cervical mucus. Some cells, however, had a less prominent fluorescent spot, probably of X-chromatin origin. This atypical body was also found in a few cervical cells of mothers of male foetuses, but was not regarded as the Y-body because of the rather indistinct morphology and the dispersed and weaker fluorescence.

In three of the sixteen women the uterine cavity was carefully perfused with 10 ml. of a sterilized balanced salt solution, and the resultant cellular component collected by centrifugation and examined for the Y-body. No Y-body was detected in these samples, though the embryos were later confirmed to be Y-positive. In one case, however, several tiny tissue fragments which appeared as clumps of cells on the slide were chorionic cells and Y-positive. Nonclumping free cells in the same slide showed no trace of the Y-body. It can be assumed that chorionic tissues were mechanically disrupted by the perfusion procedure.

The possibility of natural migration or shedding of chorionic cells into the cervical mucus early in embryonic development is, therefore, very unlikely, and clinical examination of cervical mucus in order to predict the sex of the embryo is unwarranted.

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Role of Erythrogenin from Liver and Spleen in Erythropoietin Production in the Anephric Rat

IT is well established that the kidney is the main site of the production and/or activation of erythropoietin (Ep)[1,2]. Gordon *et al.*[3] have presented evidence indicating that under both normal and hypoxic conditions the production of circulating Ep involves the interaction of a renal erythropoietic factor (also termed erythrogenin) with a serum protein component. Erythrogenin can be extracted from the light mitochondrial fraction prepared from homogenates of mammalian kidneys.

Extrarenal mechanisms of Ep production, however, seem to function in both humans[4] and rodents[5]. Recent experiments indicate that, although the capacity of anephric rodents to produce Ep in response to mild hypoxia (0.45 atm air) is gradually abolished within 12–24 h after nephrectomy[6,7], a sustained Ep production can be elicited in these animals by severe hypoxic stimuli (0.35–0.37 atm air)[8]. Intravenous administration of erythrogenin fully restores the ability of 24-h anephric rats to initiate Ep production when subjected to mild hypoxia[6] and the liver has recently been implicated as a possible source of Ep[9]. The present studies were undertaken in an attempt to verify the role of both liver and spleen as possible sources of erythrogenin in extrarenal Ep production.

In all experiments, female mice of the CF1 strain weighing 20–25 g or male rats of the Long-Evans strain weighing 150-200 g were used. The animals were maintained on a diet of pellets and tap water *ad libitum*. Levels of Ep in test materials were assessed in ex-hypoxic polycythaemic mice according to a previously reported technique[10]. Results are expressed in terms of either % ^{59}Fe incorporation into red blood cells per 48 h or equivalent units of the International Reference Preparation (IRP) of Ep.

Sham operations, splenectomy or subtotal hepatectomy were performed 12 h prior to bilateral nephrectomy. One group of sham-operated animals did not have their kidneys removed. The rats were exposed to a bout of severe hypoxia (0.37 atm air for 6 h) 13 h after the initial operation (1 h after nephrectomy). Sham-operated or nephrectomized rats maintained at room pressure were used as controls. Each group consisted of at least three animals. All operations were performed under light ether anaesthesia. Subtotal hepatectomy involved removal of both the middle and left lobes and two-thirds of the right lobe. The caudate lobe was not removed. Bilateral nephrectomy was performed by way of the ventral approach. Blood was collected by cardiac puncture under light ether anaesthesia immediately after the end of the hypoxic period. The plasma pooled from each group was dialysed against physiological saline for 24 h at 4° C and stored at −20° C until assayed.

Sham-operated or nephrectomized rats were subjected to hypoxia (0.37 atm air for 6 h) 1 h after the operation. The rats were totally exsanguinated immediately after the hypoxic period. Control animals, subjected to either sham operation or nephrectomy, were maintained at room pressure. The light mitochondrial extract (LME) from kidney, liver or spleen was prepared by a modification of the method reported by Cantor *et al.*[11] for renal LME (erythrogenin).

Serum obtained from normal male rats was dialysed against 100 volumes of 0.005 M Na_2EDTA for 24 h at 4° C. The material was redialysed against deionized water (100 volumes) for 24 h and the dialysate, normal rat serum (NRS), frozen at

Table 1 Ep Activity in the Plasma of Rats Subjected Sequentially to Sham Operation, Splenectomy or Subtotal Hepatectomy; Bilateral Nephrectomy; and Hypoxia (0.37 atm Air for 6 h)

Group No.	Treatment of donor rats	Amount of plasma injected (ml per assay mouse)	Mean % RBC-^{59}Fe incorporation ±s.e.m. in recipient assay mice	Equivalent IU Ep ml^{-1}
1	Sham operation+hypoxia	0.1	8.08 ± 1.37	2.0
2	Sham operation+nephrectomy+hypoxia	1.0	11.67 ± 0.48	0.52
3	Splenectomy+nephrectomy+hypoxia	1.0	11.21 ± 0.85	0.46
4	Subtotal hepatectomy+nephrectomy+hypoxia	1.0	2.71 ± 0.29*	0.05
5	Sham operation+room pressure	1.0	0.81 ± 0.09	ND
6	Sham operation+nephrectomy+hypoxia	1.0	0.70 ± 0.05	ND
	Standards (injected in assay mice)			
	Saline		0.51 ± 0.07	
	0.05 IU Ep		2.68 ± 0.12	
	0.20 IU Ep		8.10 ± 0.63	

*$P<0.01$ when compared with groups Nos. 2 and 3.
A minimum of three rats and six assay mice per group.
Assay in ex-hypoxic polycythaemic mice.

Table 2 Erythropoietic Activity of Light Mitochondrial Extracts (LME), incubated with either Saline or Normal Rat Serum (NRS), prepared from Kidney of Sham-operated Rats or Liver and Spleen of Nephrectomized Animals

Group No.	Treatment of donor rats	Incubation procedure of test material	Mean % RBC ^{59}Fe incorporation ±s.e.m. in recipient assay mice
1	Sham operation + hypoxia	Kidney LME + saline	1.08 ± 0.04
2	Sham operation + hypoxia	Kidney LME + NRS	4.32 ± 0.27
3	Sham operation + room	Kidney LME + saline	0.74 ± 0.16
4	Sham operation + room	Kidney LME + NRS	1.53 ± 0.19
5	Nephrectomy + hypoxia	Liver LME + saline	0.88 ± 0.06
6	Nephrectomy + hypoxia	Liver LME + NRS	3.19 ± 0.22
7	Nephrectomy + room	Liver LME + saline	0.77 ± 0.08
8	Nephrectomy + room	Liver LME + NRS	1.36 ± 0.12
9	Nephrectomy + hypoxia	Spleen LME + saline	1.03 ± 0.10
10	Nephrectomy + hypoxia	Spleen LME + NRS	3.44 ± 0.51
11	Nephrectomy + room	Spleen LME + saline	0.50 ± 0.17
12	Nephrectomy + room	Spleen LME + NRS	1.14 ± 0.05
13	None	NRS + saline	0.71 ± 0.08
	Standards (injected in assay mice)		
	Saline		0.62 ± 0.13
	0.05 IU Ep		2.09 ± 0.11
	0.20 IU Ep		5.18 ± 0.79

A minimum of three donor rats and six assay mice per group. Each mouse received a total of 2 ml of the incubation mixtures.
The rats were either exposed to hypoxia (0.37 atm air for 6 h) or maintained at room pressure. Assay in ex-hypoxic polycythaemic mice.

−20° C until used. Both liver, spleen and kidney LME were incubated with equal volumes of NRS for 45 min at 37° C in a water bath incubator with constant shaking. In control vessels, LME and NRS were incubated with equal volumes of physiological saline under the same conditions. All incubation mixtures were stored frozen at −20° C until assayed.

Table 1 shows that significant levels of Ep are detected in the plasma of nephrectomized rats subjected to severe hypoxia. This activity appears to be approximately 25–30% of that exhibited by sham-operated controls. It has been shown previously that in anephric rats weighing approximately 250 g the Ep response to hypoxia is significantly lower (10–15% that of sham-operated controls)[6,7]. No Ep activity is detected in the plasma of nephrectomized animals maintained under room pressure conditions. Although preliminary splenectomy does not modify Ep levels in the plasma of anephric rats exposed to hypoxia, subtotal hepatectomy abolishes extrarenal Ep production almost completely.

As shown in Table 2, the LME of both liver and spleen obtained from nephrectomized rats subjected to hypoxia, although by itself erythropoietically inactive, exerts a significant stimulatory effect in assay mice when incubated with NRS. A similar Ep-generating activity was detected in the LME (erythrogenin) extracted from the kidneys of sham-operated rats subjected to an equivalent hypoxic period. It is assumed that both the liver and spleen of nephrectomized, hypoxic rats show a significant level of erythrogenin activity although no Ep-generating activity was observed in the LME extracts of both organs obtained from anephric animals maintained under room pressure conditions. Thus, the correlation between the levels of Ep in the plasma and erythrogenin in the liver and spleen suggests that extrarenal Ep production is at least partially mediated by hepatic and splenic erythrogenin.

Although comparable erythrogenin activity per g of wet tissue was obtained from both liver and spleen, subtotal hepatectomy results in almost complete abolition of extrarenal Ep production, while splenectomy does not exert a significant effect. The prominent role of the liver in this phenomenon may be because of the larger weight of this organ as compared to the spleen. Gordon *et al.*[12] have presented evidence indicating that, in a patient with hepatoma associated with erythrocytosis, extracts obtained from the hepatic neoplasm served as a component for the *in vitro* generation of Ep by renal erythrogenin. Therefore, an additional hypothesis to explain the present observation assumes that the liver produces factor(s) other than erythrogenin necessary for Ep production.

The present studies indicate that Ep production in anephric rats subjected to severe hypoxia is mediated by extrarenal erythrogenin and that the liver seems to be the main functional source of extrarenal Ep production.

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Body Weight as a Criterion in Judging Bone Mineral Adequacy

APART from the extreme criterion of bone fracture, there is no definitive measurement of bone adequacy in terms of strength in relation to function. Comparison of bone mineral is usually made on the basis of age[1] or sex[2] without regard to the size or the physical status of the individual. This is in spite of Galileo's observation that the weight of a body is a function of the cube of its diameter whereas the strength of a bone is a function of the square of its diameter[3]. Galileo also conceived that a constant sized bone would support more weight if it were made of a stronger material, but did not seem to be aware that bones are subject to change in density. This is rather surprising as he discussed at some length the density of various substances. We now know that bone density varies and that the strength of both trabecular[4] and cortical bone[5] increases with density.

Integration of these observations provides an explanation of how bones of animals of various species, age, sex and position in the body maintain functional adequacy. It seems that not only would a heavier animal have proportionally larger bones, but a given sized bone could increase in density in response to increased body weight.

The experiment reported here was designed to test the effect on bone density of changing the weight borne by the bone, by applying weights to the lumbar region of sheep. Seventeen castrated male sheep were divided into two groups, and after pretreatment for 6 weeks, saddles made from hessian, with calico lined pockets, were placed over the loins of both groups for 7 weeks. Nine Merino wethers each carried saddles with 10 kg (which approximates full term twin foetal weight) of cast iron in the pockets and the control group of eight wethers carried wood shavings of comparable bulk in their saddles. The sheep were housed randomly in two enclosed stalls each of 12 m² floor area, and fed freely on lucerne and wheaten chaff, with cotton seed meal added at the rate of 30 g per sheep per day. The control sheep made an average liveweight gain of 3.68 kg during the experiment and weighted sheep 2.27 kg. Observations were made weekly, when total weight, γ-ray absorption of the tibial tarsal bone and the leg thickness (medio-lateral) over the tibial tarsal bone were recorded.

Estimation of γ-absorption was made by passing a collimated beam of radiation from ^{241}Am through the tibial tarsal bone by a self-locating cast placed on a sheep's tarsus, and the emergent radiation was read on γ-detecting equipment (Nuclear Enterprises Ltd scintillation counter DMI-1 and a variable energy level detecting spectrometer NE 8433) (our unpublished results). The thickness of the leg (bone and overlying soft tissue) through the tibial tarsal region was measured using dial-faced callipers. The absorption was calculated as

$$\mu t = 2.303 \log_{10} (I_0/I_t)$$

where μ is the absorption coefficient of the principal wavelength of ^{241}Am; t the thickness of leg across the tibial tarsal bone (cm), and I_0 the intensity of radiation through air and I_t the intensity of radiation through t.

Figure 1 shows the mean values of γ-absorption (μt) for the weighted and control sheep in relation to time. There was little

Table 1 Comparison of Mean Changes in Absorption of Radiation (μ, μt and μt^2) between Periods in Nine Weighted (W) and Eight Control (C) Sheep

Comparison (periods)	$\Delta\mu.10^2$ W	$\Delta\mu.10^2$ C	$\Delta\mu t.10^2$ W	$\Delta\mu t.10^2$ C	$\Delta\mu t^2.10^2$ W	$\Delta\mu t^2.10^2$ C
2–1	1.06	1.16	0.94	1.38	−5.54	−2.64
	t=0.16		t=0.26		t=0.57	
3–1	1.91	0.55	5.00	1.20	11.77	1.19
	t=2.28*		t=2.41*		t=2.07	
4–1	1.61	0.33	4.10	0.28	8.96	−1.80
	t=2.92*		t=4.55‡		t=2.17*	
3–2	0.85	−0.62	4.10	−0.18	17.31	3.88
	t=2.45*		t=2.44*		t=2.50*	
4–2	0.56	−0.63	3.18	−1.11	14.50	−0.84
	t=3.05†		t=3.31†		t=3.20†	

Each period represents the average of the mean of three successive weekly readings on each sheep. No sheep carried weights during periods 1 and 2; in periods 3 and 4 the weighted sheep carried 10 kg slung across their loins.

*, $P<0.05$; †, $P<0.01$; ‡, $P<0.001$; $df=15$.

change during pretreatment but there was a rapid increase in γ-absorption in the weighted group to a maximum within 2 weeks of applying the extra weight. Because of large individual differences among the sheep, no significant differences between control and weighted groups could be detected by Student's t test at any time. Sheep in the treatment group apparently resented the weighted saddles and ran about excitedly when not physically restrained, while control animals showed evidence of a small increase in bone density immediately after the saddle was applied, possibly as a result of change in exercise pattern. More uniform readings were obtained in the later weeks when the animals became more placid, so tension on bone as a result of exercise could be important in influencing bone density measurements.

To test if the performance of the animals changed significantly as a result of the different treatments, the data were grouped into four periods of 3 weeks each as shown in Fig. 1. Periods 1 and 2 represented weeks 1–3 and 4–6 respectively (before the weights were applied), and periods 3 and 4 represented weeks 8–10 and 11–13 respectively. Week 7, being a transition week, was omitted from the grouping. The mean value for each animal within each period was obtained, and the changes for each animal between periods calculated. These mean values for changes in μt for control and weighted groups are shown in Table 1 together with the significance of differences between these means. No difference between the mean changes in the weighted and control groups between periods 1 and 2 was detected, but the changes from periods 1 and 2 to 3 and 4 are significantly greater in the weighted group than in the control group. A very highly significant difference between the change in γ-absorption of weighted and control groups occurred between periods 1 and 4.

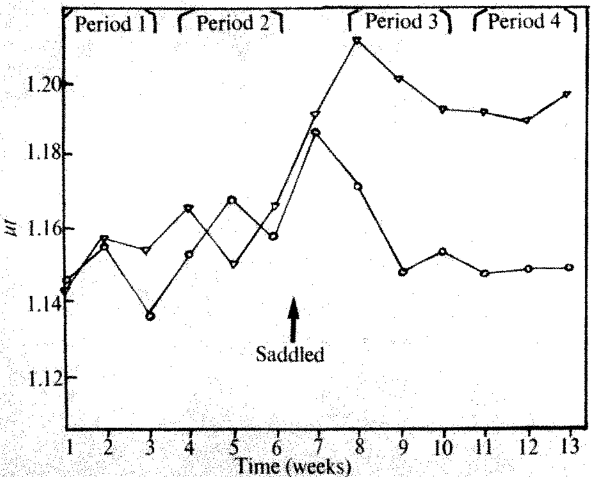

Fig. 1 The γ-ray absorption (μt) of eight control (○) and nine weighted (▽) sheep carrying 10 kg from week 6.

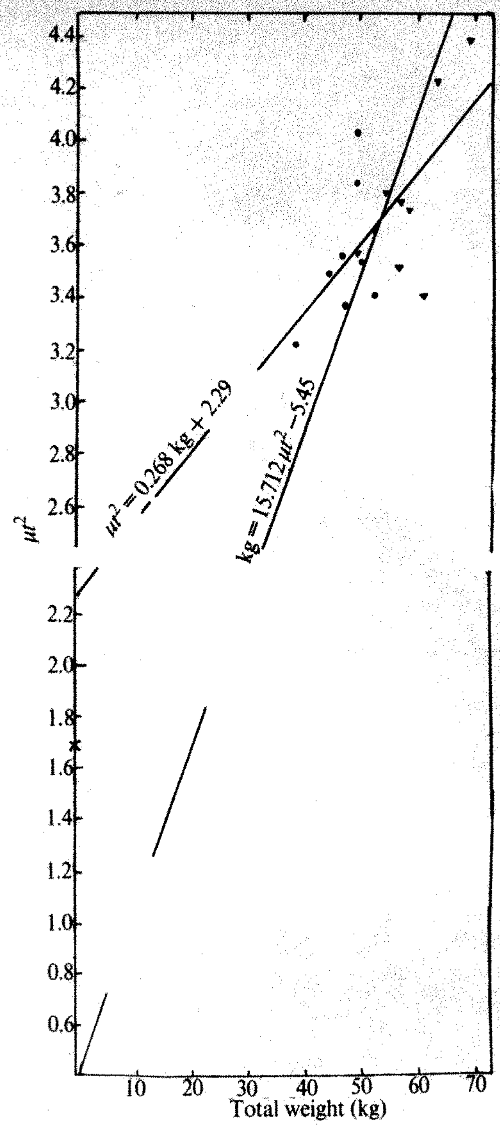

Fig. 2 Association between bone mineral (μt^2) of sheep tibial tarsal bones and the total weight of sheep (including saddles) after carrying saddles for 7 weeks. The μt^2 for tendon (X) is shown. ●, Control; ▼, weighted sheep, r=0.65.

Changes in leg thickness could account for changes in γ-absorption, but this is unlikely as the derived absorption coefficients (μ), which are independent of t, show differences of similar significance. Moreover, no significant changes in t were detected throughout the experiment. Unpublished work in this laboratory shows that there is a linear relationship between μ and the specific gravity of whole tibial tarsal bones of sheep (r=0.84, $P<0.001$). It seems then that the changes in γ-absorption are a result of changes in specific gravity of the bone rather than changes in leg size.

In order to estimate the total mineral in the leg, μ was multiplied by t^2, that is, a density measurement times an estimated area across the leg, and designated μt^2. These results are also shown in Table 1. The correlation between μt^2 and total weight at the thirteenth week is illustrated in Fig. 2 (r=0.65, $P<0.01$). Both regression lines are shown together with a point X on the y axis which is the μ for tendon (unmineralized collagen) multiplied by the square of the average leg thickness; this lies between the two regression lines.

Clearly, an increase in body weight produces a rapid increase in bone density, and the correlation between body weight and bone density indicates that the forces applied to a bone, whether they be in the form of weight or tendon pull, determine the density of bone in individual animals in a relatively uniform manner. If this relationship holds for larger numbers of animals and is consistent across species, body weight must be regarded as an essential variable when judging bone adequacy. It should find application in assessing bone dystrophy and its treatment, bone mineral reserves in various physiological states such as pregnancy and lactation, and the adequacy of bone response during an exercise programme.

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Variation in Trace Metal Concentrations along Single Hairs as Measured by Proton-induced X-ray Emission Photometry

Trace elements in human and animal hair have been studied using spectrophotometric, atomic absorption, emission spectrographic, neutron activation, and spark source mass spectrometric methods. We report here measurements of trace elements in human hair using the technique of proton induced X-ray production.

Twenty-seven elements have been identified in human hair[1] in concentrations ranging from 0.1 to 100 μg^{-1}. Early measurements indicated a wide range of concentrations of almost all trace elements for different individuals but in certain cases correlations with sex, age, and colour of hair were reported[2-6]. It has been assumed that hair may be a good tool for studying the storage of nutrients by the body as it accumulates over a period of time and is relatively inert metabolically. Efforts to relate trace element content of hair to diseases which are known to influence trace elements in other parts of the body were not successful[7-11]. Several research groups[12-17] studied the level in hair of trace elements which could possibly be hazardous to health.

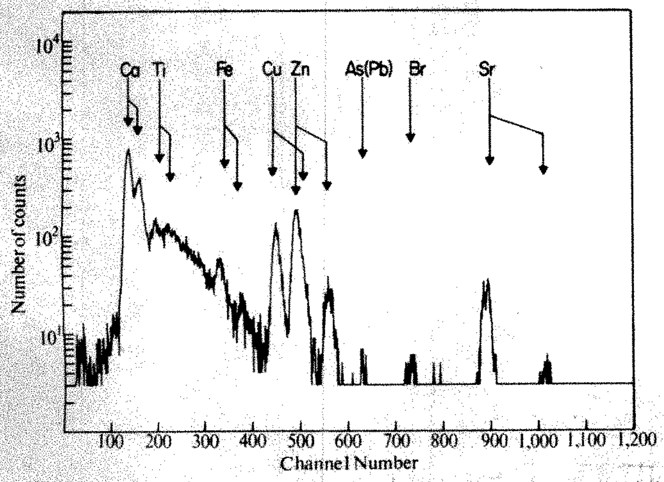

Fig. 1 X-ray spectrum obtained by exposing a single hair sample to a 3 MeV proton beam.

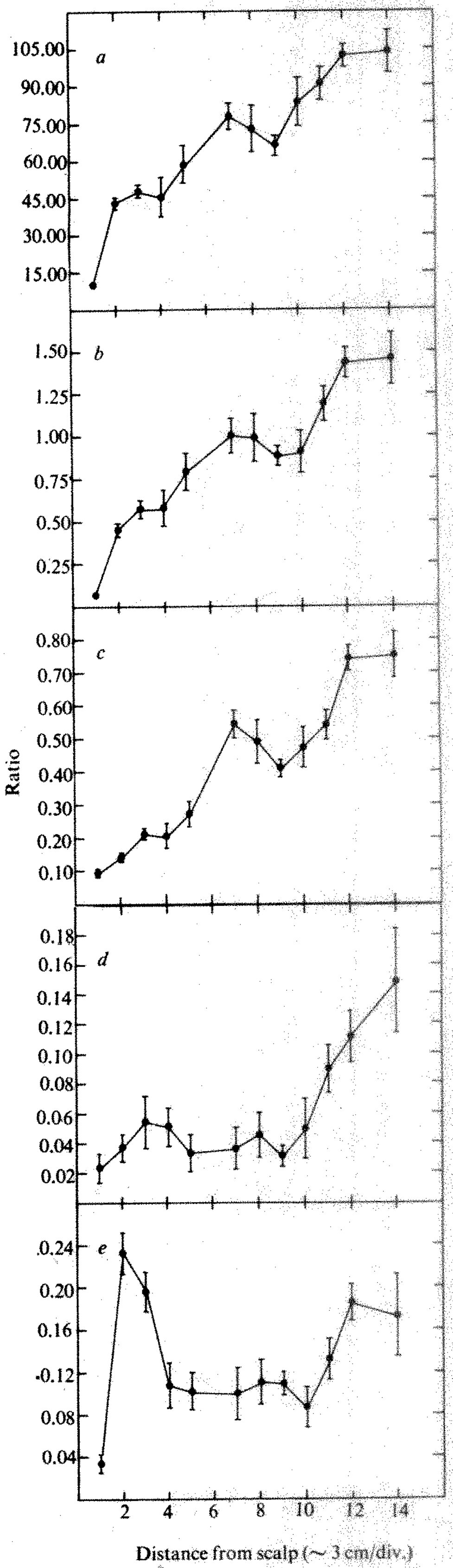

Fig. 2 Ratios of the element concentrations Ca/Zn (*a*), Sr/Zn (*b*), Cu/Zn (*c*), Br/Zn (*d*) and Fe/Zn (*e*), as a function of distance along the hair.

In the measurements reported here a single hair from a female head has been washed in triply distilled water, and cut into fourteen pieces approximately 3 cm in length. Each sample was then fixed to an aluminium frame. Each target was exposed to the beam of 3 MeV protons from the Rice University tandem Van de Graaff accelerator. Detector efficiency for elements lighter than Ca and the low energy bremsstrahlung background was artificially reduced by introducing a polystyrene absorber in front of the detector. The resulting X-rays were detected by a Si(Li) detector and amplified, then processed and stripped for peak intensity by an IBM-1800 computer. Figure 1 shows the X-ray spectrum obtained for one of the samples. Peaks associated with Ca, Ti, Fe, Cu, Zn, Pb, Br, and Sr K_α and K_β X-ray lines are present in all spectra.

The ratios of elements in the hair were normalized to ratios obtained from targets with known element concentrations. Figure 2 shows the ratios Ca/Zn, Cu/Zn, Sr/Zn, Fe/Zn, and Br/Zn as a function of distance along the hair from the scalp. The Ca/Zn, Cu/Zn, Sr/Zn ratios are found to increase monotonically with length along the hair except for a small dip in the 9–10 region. The Br/Zn and Fe/Zn ratios show a more complex structure, but also display a dip in 9–10 region. The Fe/Zn ratio in particular, undergoes very strong fluctuation near the base of the hair.

Two additional measurements were performed with beam-resistant and multihair targets using hair from the same female head. All three measurements were consistent. In addition, the shampoo which had been used in regular washing of the hair was found to contain no significant concentrations of metals heavier than Ca.

These results show that the concentrations of trace elements undergo large fluctuations in close proximity to the scalp. The relative concentrations of Ca, Sr, Cu, Fe, Br, and Zn change significantly with distance along the hair. The source of these variations may be associated with the environmental and biomedical history of the hair and its owner.

These facts must be considered when evaluating previous measurements of trace elements in hair. It is possible that some reported inconsistencies are due to the variation of element concentrations along single hairs and, therefore, future studies must pay attention to this problem.

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Chemical Exploration of the Microhabitat by Electron Probe Microanalysis of Decomposer Organisms

INCREASING emphasis on the role of microorganisms as nutrient concentrators in ecosystems calls for the accurate, reliable chemical analysis of these cells within their microhabitats. The inability to perform such analyses has been a principal limitation of existing macrochemical techniques. Witkamp[1], Stark[2], and Todd and Cromack[3] have demonstrated that terrestrial microflora can concentrate calcium, potassium and magnesium to a point that each becomes a major nutrient pool. Microarthropods—important grazers of microflora—link the terrestrial detritus-based food chain to other chains. This paper outlines a technique which enables the chemical composition of the microfloral and faunal biomass to be analysed without its destruction or separation from the detrital matrix. With the exception of laboratory bacterial cultures, all samples were collected from the US Forest Service Coweeta Hydrologic Laboratory field site, North Carolina.

Scanning electron microscopy is currently being used to provide "visual" observations of microorganisms in terrestrial[4–6] as well as estuarine ecosystems[7]. Electron probe microanalysis utilizes a similar, focused high-energy beam of electrons to excite the constituent atoms of a sample to emit characteristic X-radiation. This radiation can be analysed and displayed as a scanned image of elemental concentration in the specimen or, in the static mode, quantitative spot analyses can be made by comparison with suitable standard samples. (With our instrument both were possible in the same run.)

The principles of electron microprobe analysis are discussed in detail by Birks[8]. He states that 1 μg samples can be analysed with one part per billion resolution for the heavier elements (atomic number greater than 12). The precision and resolution, however, depend greatly on the nature of the sample and its preparation. Impregnation with plastic or metal, polishing to a smooth, flat surface, and vacuum-coating with carbon have usually been considered necessary. In this study, however, useful quantitative as well as qualitative data were obtained using the less disruptive preparation techniques outlined by Todd *et al.*[7] for observing biological samples in the scanning electron microscope.

For examination and analysis, our specimens were affixed by double adhesive cellulose tape or silver paint to the surface of a specimen stub. The air-dried specimens were then coated (200–400 Å) with a gold-palladium alloy by vacuum evaporation. Secondary electron images were observed using a Cambridge Stereoscan Electron Microscope (Mark II A, Cambridge Instruments Co., London, England) operated at an accelerating voltage of 10 kV. X-ray and backscattered electron images as well as spot analyses were made with an electron microprobe analyser (Model 400-S, Materials Analysis Co., Palo Alto, California) operated at 20 kV accelerating voltage with 0.02 μA sample current. Both secondary electron and X-ray emission images were recorded using Type 55 P/N Polaroid sheet film (Polaroid Corp., Cambridge, Massachusetts).

Comparisons between X-ray and secondary electron images

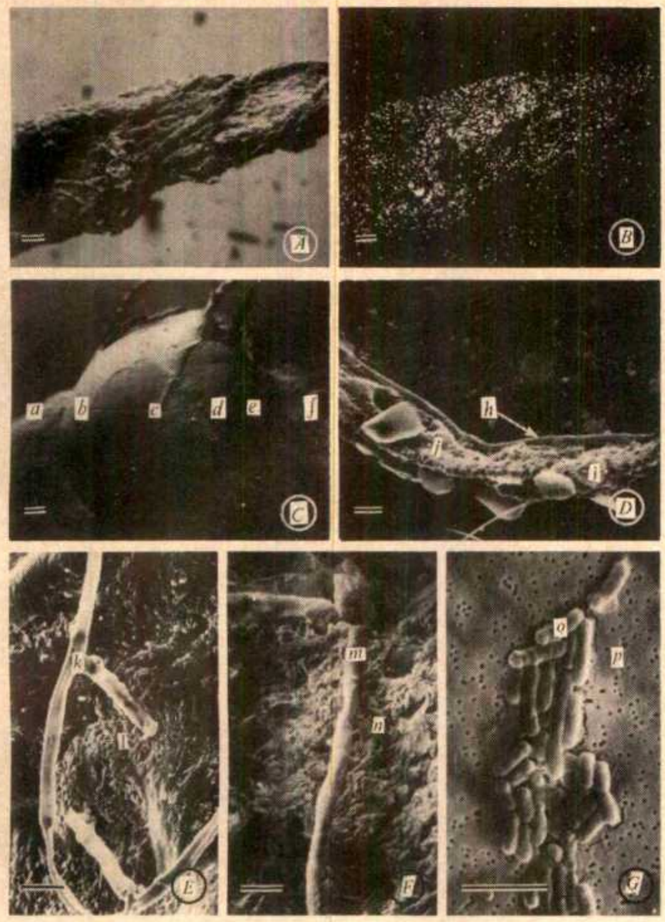

Fig. 1 *A* and *B* are electron microprobe analyser pictures of a rhizomorph sample isolated from decaying deciduous forest litter. A 100 μm marker is indicated at lower left. *A*, Image made by backscattered electrons. *B*, Calcium X-ray image. *C*, Galumnidae mite for which calcium values were determined after electron bombardment at each indicated point (*a* and *f* are background points) for 10 s periods at 20 kV. Calcium values (mg g^{-1}) for the points are: *a*, 1.5; *b*, 10.6; *c*, 18.9; *d*, 31.5; *e*, 12.7; *f*, 2.5. *D–G* are scanning electron micrographs of representative assay fields. *D*, White pine needle fragment (*g*) from litter with adhering insect wing scales (*h*) and a fungal hypha (*j*). *E*, Oak leaf (*l*) taken from decaying litter with a fungal hypha (*k*). *F*, Fungal hypha (*m*) in soil (*n*) underlying decaying oak litter. A 40 μm marker is indicated at lower left of each field. *G*, *Bacillus subtilis* cells (*o*) concentrated on the surface (*p*) of a membrane filter. A 10 μm marker is indicated at lower left.

were used to estimate the elemental content of a variety of biological specimens. These analyses have included a study of calcium, potassium, and magnesium distribution in organisms inhabiting decaying, mixed deciduous forest litter. Quantification of this technique is possible with macrochemical assay procedures such as emission spectroscopy and atomic absorption spectroscopy[3]. For example, the fungal rhizomorph illustrated in Fig. 1*A* and *B* contains 29.7 mg g^{-1} Ca as determined by these procedures. These counts can be converted to elemental values by comparison with standards of known concentrations. A calcium value of 134 $\mu g\ g^{-1}$, potassium 194 $\mu g\ g^{-1}$ and magnesium 140 $\mu g\ g^{-1}$ is approximately equal to a respective X-ray emission value of one count every 10 s.

A quantitative application of microprobe analysis to biological material is illustrated in Fig. 1*A–G* and Table 1. Fig. 1*A* is a back-scattered electron micrograph of a rhizomorph sample, and Fig. 1*A* is a calcium X-ray image of the same rhizomorph. Fig. 1*C* depicts a whole body of a Galumnidae mite, a common litter-inhabiting organism which grazes on fungi. Point analysis counts, which resulted in minimum distortion of the sample, were made at each point along a transect (*a–s*).

An application of this technique which enables nutrients to be analysed in a variety of biologically different samples located close to each other is illustrated in Fig. 1*D*. A freshly collected sample from the "L" layer of litter in a white pine stand was examined and comparison was made of the magnesium, calcium and potassium contents of a pine needle fragment, an insect wing scale and a fungal hypha, all situated within 1 mm (Table 1). All three samples contain the same relative amounts of magnesium and potassium. The calcium content of the insect wing scale, however, is eight times higher than that found in the pine litter. There is twice as much calcium in the fungal hypha as in the decaying pine needle.

Microprobe comparisons between a fungal hypha growing on a deciduous leaf and one growing in soil (Fig. 1*E* and *F*) are summarized by the numerical values for the magnesium, calcium and potassium content of these two samples in Table 1. There is no significant difference in the magnesium content of the fungal hypha colonizing the oak leaf or soil substrates. The calcium content of the fungal hypha is, however, twice that of the oak leaf, although there is no difference between the amount of calcium in the hypha and soil. Potassium seems to be concentrated within the fungal biomass of both microhabitats.

Chemical analysis of a specimen as small as an individual bacterial cell can be accomplished by the microprobe procedure, although samples of this size approach the 1 μm limit of resolution of the microprobe beam and are extremely "thin" with respect to the effective electron penetration. A stock culture of a common soil bacterium, *Bacillus subtilis*, was grown on a complex medium and the collected cells concentrated upon the surface of a 'Nuclepore' membrane filter[9]. Spot analyses were made of both the calcium and magnesium contents of the bacterial cells within the same field shown in Fig. 1*G* and are listed in Table 1. The calcium content of a *Bacillus subtilis* cell is estimated by the microprobe method as 1.1% and compares favourably with the 0.8% determined by emission spectroscopy for a mixed bacterial population grown under similar conditions[3].

Electron probe microanalysis can be used to determine concentrations of essential nutrients in biological components of the terrestrial decomposer food web, such as the detrital substrate, microbial population and microbial grazers. As decomposer organisms are of great importance in cycling of nutrients in ecosystems[10], any new technique which would give better estimates of nutrient pools contained within the decomposer food web would add substantially to the understanding of functional processes within ecosystems. Current modelling efforts being devoted to description and prediction of ecosystems by computer simulation would benefit from more accurate estimates of nutrient pools in decomposer organisms.

Several distinct advantages of this technique are evident when comparisons are made with existing macrochemical procedures. Examination of a variety of biologically different specimens located close to each other with minimum distortion of sample is possible. With the scanning electron microscope even a small specimen can be observed and uncontaminated areas can be selected for examination. Not only can estimates of microbial biomass be made from quantitative sampling techniques but it is also possible to ascertain the distribution of nutrients within the organism itself. As the specimen is not destroyed in the analytical procedure, assays of a given sample can be repeated to obtain statistical estimates of the nutrient content.

The application of this technique is not limited to investigations concerning terrestrial decomposer organisms, but will find application in any situation in which microorganisms play an integral part. The use of electron probe microanalysis will enable assessment of the role that decomposer organisms play in nutrient cycling.

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Table 1 Nutrient Analyses of Fungal and Bacterial Biomass shown in Fig. 1*D*-*G*

Specimen	No. of assays	Elemental content (mg/g) Magnesium	Calcium	Potassium
Fig. 1*D*				
Fungal hypha (*h*)	11	3.02±0.32* †	29.60± 2.48*	22.36±1.84
Insect wing scale (*i*)	6	2.47±0.25	112.58±14.84*	18.95±0.85
White pine needle (*j*)	7	1.72±0.20	14.56± 4.31	18.37±1.07
Fig. 1*E*				
Fungal hypha (*k*)	8	2.74±0.25	81.64± 4.67*	17.48±0.46*
Oak leaf (*l*)	5	3.30±0.36	44.62± 8.21	13.23±1.07
Fig. 1*F*				
Fungal hypha (*m*)	8	2.66±0.43	66.07± 6.95	46.21±2.81*
Soil (*n*)	4	3.66±0.98	65.15±17.66	19.90±7.46
Fig. 1*G*				
Bacteria (*o*)	12	0.08±0.08	11.32± 2.41	No data

Values reported were determined from X-ray emissions after electron bombardment of each specimen for several 10 s periods at an accelerating voltage of 20 kV.

* Indicates significant difference ($P<0.05$) in elemental content between sample and substrate (pine needle, oak leaf, soil).

† Standard error of mean is given for all samples.

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Role of Protozoa in Waste Purification Systems

Pirt and Bazin[1] discussed possible adverse effects of bacterial predation by protozoa on the removal of substrate by activated sludge. Their analysis considered not activated sludge but a single-stage chemostat without recycle as used experimentally by Curds[2], and mathematically by Curds[3] and Canale[4], for the study of predator/prey interactions. Pirt and Bazin[1] concluded that efficient waste removal could best be achieved with two chemostats in series, eliminating protozoa from the first stage by operating it at a dilution rate in excess of their maximum specific growth rate.

Table 1 compares the concentrations of bacteria, protozoa, and substrate obtained from this system with those from a single-stage chemostat having the same overall residence time, demonstrating the better performance of the two-stage system. Table 1 also shows the effluent concentrations for a two-stage system of the same overall residence time which allows growth of protozoa in both stages. Contrary to the suggestion of Pirt and Bazin[1] the presence of protozoa in both stages improved treatment, with a further reduction in total solids concentration.

These results were obtained by assuming, as did Pirt and Bazin[1], that all the bacteria were dispersed and therefore

Table 1 Calculated Concentrations of Bacteria, Substrate and Protozoa in Effluent from Single-stage and Two-stage Continuous Culture

Single-stage operation

Dilution rate (h^{-1})	Specific growth rate (h^{-1}) Bacteria	Protozoa	Effluent concentration (mg l^{-1}) Substrate	Bacteria	Protozoa
0.225	0.9989	0.225	8,858	128	221

Two-stage operation

Stage 1				Stage 2					
Dilution rate (h^{-1})	Concentration (mg l^{-1})			Dilution rate (h^{-1})	Specific growth rate (h^{-1})		Concentration (mg l^{-1})		
	Substrate	Bacteria	Protozoa		Bacteria	Protozoa	Substrate	Bacteria	Protozoa
0.9	90	4,955	0	0.3	0.045	0.3	0.47	300	2,350
0.385	29.6	2,566	1,209	0.541	0.039	0.27	0.40	204	2,393

(μ_{max} bacteria = 1.0 h^{-1}, K_s bacteria = 10 mg l^{-1}, μ_{max} protozoa = 0.4 h^{-1}, K_s protozoa = 100 mg l^{-1}, Y bacteria = Y protozoa = 0.5, S_0 = 10,000 mg l^{-1}, residence time 4.4 h).

available for predation[5]. Less than 10% of the bacteria in activated sludge are freely dispersed[6], however, and those within the floc are relatively immune to predation. Fig. 1 shows the steady-state values obtained for the concentrations of bacteria, substrate, and protozoa in a single stage when another bacterium, using the same substrate but growing as a floc, is present. It was assumed that such a bacterium would grow at a slower rate than that of a dispersed organism, and the growth constants were chosen so that, in the absence of predation, it would be washed out of the system. In the three-component system washout of the protozoa occurred at the same dilution rate (>0.39 h^{-1}) as in the two-component system[1], whereas the floc-forming organism was washed out when the dilution rate exceeded 0.38 h^{-1}. The presence of flocculated bacteria considerably reduced the effect of predation on substrate concentration (Fig. 1).

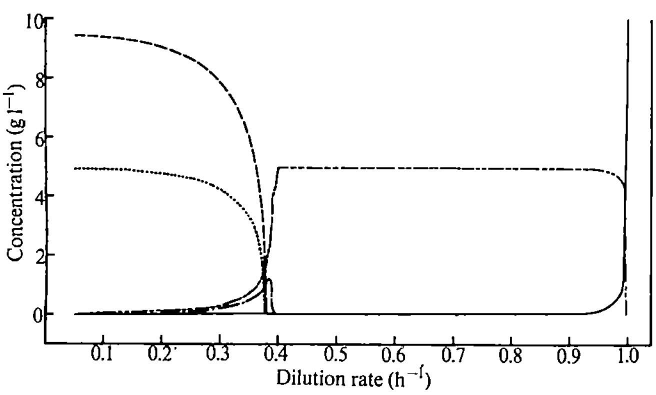

Fig. 1 Steady-state values of a prey, predator, and flocculated bacterium in a chemostat. Initial concentration of substrate, 10 g l^{-1}.

	μ_{max} (h^{-1})	K_s (mg l^{-1})	Y
Dispersed bacteria	1.0	10	0.5
Flocculated bacteria	0.5	20	0.5
Protozoa	0.4	100	0.5

Substrate (2-component system[1]), - - -; Substrate (3-component system), ——; dispersed bacteria, — . . —; flocculated bacteria, ...; protozoa, — - — .

The requirement of waste treatment is to reduce the concentration of substrate (soluble organic compound or dispersed bacteria) as far as possible, thus limiting the extent of biological action when the effluent is discharged to the environment. It follows that within a biological process the microorganisms will be subject to severe substrate limitation and will have very low specific growth rates (Monod[7]; $\mu = \mu_{max}s/(K_s + s)$). In the activated sludge process the critical stage is the settlement and recycling of the biomass so that the specific growth rate of the organisms is much less than the dilution rate[5,8-10], a situation similar to the second stage of a two-stage chemostat system[11] (Table 1). As settlement depends on the presence of flocculating bacteria, the activated-sludge process combines the advantages of a specific growth rate less than the dilution rate with the dominance of bacteria resistant to predation (Fig. 1). Additionally, attached forms of protozoa develop[5] which settle and are recycled: these have a specific growth rate similar to that of the recycled bacteria and, for the same residence time, will reduce the concentration of dispersed bacteria in the final effluent to levels below those obtainable with the two-stage chemostat system. Thus, in the activated-sludge system the concentrations of both substrate and dispersed bacteria are reduced to a low level, the effect of predation on substrate concentration is minimized, and the role of protozoa becomes chiefly that of effluent clarification[5].

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Resistance of *Raphanobrassica* to Clubroot Disease

THE classical amphidiploid *Raphanobrassica* ($2n = 36$, genomic formula *rrcc*), first reported by Karpechenko[1], was of considerable academic interest and appears in many textbooks. As far as is known *Raphanobrassica* does not occur in the wild and it has not been exploited practically.

An attempt is being made at Pentlandfield to develop *Raphanobrassica* as a new forage crop from autotetraploid forms of fodder radish (*Raphanus sativus* L., $2n = 36$, *rrrr*) and fodder kale (*Brassica oleracea* L., $2n = 36$, *cccc*). The possibility of clubroot resistance superior to that of rape (*B. napus* L. ssp. *oleifera* (Metzg. Sinsk.)) is an important consideration in this programme. That this might be possible is suggested by the fact that four cultivars of fodder radish proved completely resistant to four physiological races of the fungus *Plasmodiophora brassicae* Woron. isolated in Wales (Johnston, personal communication). Fodder kales are generally accepted as possessing at least some resistance, although little critical work has been carried out.

A number of *Plasmodiophora* cultures were obtained from farm crops in south and east of Scotland. Spores were extracted by the method described by Macfarlane[2] and stored at 2±1° C in the dark. Spore numbers were assessed with the aid of a haemocytometer and diluted to a constant concentration of 10^7 spores ml^{-1}.

Various forms of *B. napus* (Table 1) have been used as differential hosts by Johnston[3] and Paterson[4] to identify the five biotypes of *Plasmodiophora* so far isolated in the UK. Seedlings of these differentials were grown to the cotyledon stage, their roots were dipped for 3 min in shallow dishes of spore suspension and they were then planted at 5×5 cm spacing in plastic gravel trays containing John Innes No. 2 compost (*p*H 6.2). Soil was kept uniformly moist and maintained at 20±3° C. Seedlings treated with different cultures were isolated from each other in separate trays. Air temperature did not fall below 8° C and plants were grown under a regime involving a "day" of 16 h.

Fifteen cultures were tested and varying reactions of the differential hosts obtained. Six cultures were found to infect heavily all five differential hosts.

Table 1 Reaction of *Brassica napus* and *Raphanobrassica* to Two Cultures of *Plasmodiophora*

	Numbers of infected seedlings in each infected class							
	Culture 1				Culture 2			
B. napus	None	Slight	Moderate	Severe	None	Slight	Moderate	Severe
"Giant" rape	—	—	—	10	—	—	—	10
Selection ex "Giant" rape	–	—	1	9	3	—	—	7
"New Zealand club root resistant" rape	2	—	—	8	2	—	—	8
"Wilhelmsburger" swede	—	—	3	7	2	—	—	7
"Nevin" rape	2	1	2	5	2	—	—	8
Raphanobrassica								
Family 1	10	—	—	—	7	2	1	—
Family 2	10	—	—	—	7	—	—	3
Family 3	10	—	—	—	9	1	—	—
Family 4	10	—	—	—	8	—	2	—
Family 5	10	—	—	—	10	—	—	—

In a second experiment, five F4 *Raphanobrassica* families were tested against two cultures giving the above reaction. These families were fairly closely related because sterility of Fl plants[5] had much reduced the genetic base of subsequent generations.

Ten seedlings of each *Raphanobrassica* family and each differential host were treated. Seedlings were randomized in rows of five in each of two trays for each culture. After 6 weeks roots were scored for degree of club formation as an indication of the level of susceptibility. Results are shown in Table 1.

It is possible that cultures attacking all five differential hosts were actually aggregates of two or more races. It would require subculturing and/or a wider range of differentials to isolate these races. The fact that the *Raphanobrassica* families gave slightly different reactions to the two cultures tested is in accord with this premise.

Clubroot resistance in *Raphanobrassica,* not present in "Nevin", the most resistant rape cultivar commercially available, is clearly shown. The extent of this resistance remains to be explored but there are grounds for optimism that, should fertility problems be resolved, a useful new forage plant may eventually result.

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Gestation Period and Birth Weight of *Australopithecus*

Based on my estimates[1] of the foetal size at birth of *Australopithecus africanus* and *A. robustus,* Frazer[2] has calculated gestation periods to be 257 and 300 d, respectively. Calculations are based on the assumption of a constant foetal growth rate of 0.06 in higher primates. Regression analysis of gestation periods and birth weights in pongids and modern man (Table 1) indicates, however, that these two factors have a low correlation ($r=0.50$, compared to $r=0.98$ for the correlation between maternal and foetal (birth) weight of higher primates[1]). This indicates that among higher primates the foetal growth rate is anything but constant, and therefore estimates on gestation periods based on this rate and birth weight are useless.

Table 1 Birth Weight and Gestation Period in Pongids and Modern Man*

Species	Mean birth weight (g)	Mean gestation period (d)
Pongo pygmaeus	1,500	275
Pan troglodytes	1,600	225
Gorilla gorilla	2,000	268
Homo sapiens	3,300	280

* Based on published data[1,3].

All that can safely be said about the gestation period of australopithecines is that its length had to be of the magnitude of that of pongids and modern man, and definitely shorter than 300 d as this has been proposed for *A. robustus.*

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Reference Abbreviations

From September 1 it is *Nature's* intention that all abbreviations of references should conform to the style of the *World List of Scientific Periodicals,* fourth ed. (Butterworth, 1963–65). The changeover will be gradual and authors submitting manuscripts from now on are asked to ensure that the references are written appropriately.

BOOK REVIEWS

Monumental Prehistory

The Chambered Tombs of Scotland. By Audrey Shore Henshall. Volume 2. Pp. 656+38 plates. (Edinburgh University: Edinburgh, 1972.) £15.

WITH the publication of volume 2 of *The Chambered Tombs of Scotland* Audrey Shore Henshall has brought to fruition nearly two decades of intensive survey and study. These tombs undoubtedly rank as the most numerous and impressive instances of early architecture in Britain: although some 5,000 years old, a number of them still stand virtually complete, and can be viewed today in a condition very close to that in which they were left by their makers, which is probably more than can be said for any Roman or Saxon structure in these islands.

The tombs discussed in this second volume, which covers western and southern Scotland, are two hundred in number. They are divided into three groups (Bargrennan, Clyde and Hebridean, plus long cairns), supplementing the six groups in northern and eastern Scotland discussed in volume 1. They are listed systematically by county, each with a plan (often by the author herself), with a full description and, where the gravegoods are preserved, with drawings of all the finds. This is undoubtedly one of the most monumental achievements in the whole field of British archaeology: it will still be an indispensable work of reference a century hence.

For the scholar today the interest does not lie solely in the great mass of data presented in the corpus. Miss Henshall's discussion of the origin and development of the tombs has been eagerly awaited, particularly because the full impact of radiocarbon dating has been felt since the publication of her first volume. The longer chronology now makes clear that many of these tombs are composite monuments, the result of several phases of construction and of reconstruction. This realization makes the question of origins a little clearer, and it is no longer necessary to seek a separate overseas origin for each of the nine groups identified. An early phase of building is now suggested in south-west Scotland, before 3000 BC, related no doubt to analogous developments in Ireland, with small rectangular burial chambers inside small cairns. In phase two the first passage graves—simple polygonal chambers with an entrance passage—were built. The story from then on is one of increasing local elaboration culminating in the construction of the impressive tombs of Caithness and Orkney, where the masterpiece of Scottish neolithic architecture, Maes Howe, may reflect further contacts with Ireland.

Some elements of this sequence are hypothetical, but the important and progressive shift is to see these developments in essentially local terms, although not divorced from a broader European context. The way is now open for more sustained locational studies, rather than the detailed typological treatments which have sometimes in recent decades become too elaborate.

Miss Henshall rightly emphasizes that in nearly all cases the gravegoods found in these tombs relate only to the final phases of their use, often centuries after their original construction. In many areas we are left with the paradoxical situation that the only archaeological evidence from the makers of these impressive monuments lies in the tombs themselves, with no artefacts or indications of domestic settlement to supplement them. It is to be hoped that this is a situation which archaeology will eventually rectify. But meanwhile we are indeed fortunate that the tombs themselves are presented here so comprehensively. The Edinburgh University Press has again produced the volume very handsomely.

In his foreword to the first volume, Professor Stuart Piggott hailed "Miss Henshall's achievement . . . a major and outstanding contribution, of solid and enduring worth, to British prehistoric studies". The publication of the second volume confirms this verdict: it is surely the most substantial contribution made for several decades to the study of British prehistory.

COLIN RENFREW

Reptiles and Amphibians

Herpetology. By Kenneth R. Porter. Pp. xi+524. (W. B. Saunders: Philadelphia, London and Toronto, 1972.) £6.60 ; $15.50.

PROFESSIONAL herpetologists have long despaired of there ever being published an authoritative text that would meet their needs. The high standard achieved by the contributors to the recently published volumes in the *Biology of the Reptilia* and the informative *Life of the Reptiles* has gone a long way towards satisfying workers on that class of vertebrates but those concerned with amphibians still lacked any textbook more recent than Noble's 1931 *Biology of the Amphibia.* Kenneth Porter's *Herpetology* seems destined to be the book that has been hoped for. It has remarkably wide coverage and reflects organizing skill and a prodigious amount of labour in sifting the relevant literature, yet the mass of information is contained in a manageable size.

The contents include sections on origins and phylogeny with generous reference to palaeontological material and characterizations to family and subfamily level, zoogeography, moisture and temperature relations, colour change, feeding, isolating mechanisms, population dynamics and man's interactions with reptiles and amphibians; about two hundred pages are devoted to functional morphology and reproductive adaptations. A list of references follows each section.

The systematic treatment follows conservative lines and generally incorporates the arguments and the changes that have been presented by the leading workers in the field but surprising omissions are both McDowell's and Miller's convincing evidence that the Dibamidae and the Anelytropsidae are congeneric and Gasc's discussion of the family's affinities. But on the whole there are reasonably few errors of omission and the text is commendably free from typographical errors. The subject index would benefit from expansion: I had to engage in mental gymnastics tracking down topics such as the lateral line system and autotomy. Another minor defect that will no doubt be rectified in a second edition lies in the use of illustrations reproduced from books originally published many years ago with captions bearing out of date names that appear nowhere in Porter's text.

It is sobering to find in the section on leading centres and British herpetologists that the author rates highly only three workers, two of whom are dead. Despite liberal quotations from G. L. Underwood's and I. Griffiths's works, the British nationality of these authors is not acknowledged. But not even this slight can detract from the value and usefulness of this highly scholarly treatise which can be enthusiastically recommended to all who are interested in herpetology.

A. G. C. GRANDISON

A Model of Development

Science Policy and Development: The Case of Israel. Edited by E. Tal and Y. Ezrahi. (Israel–Latin American Symposium on Science Policy and Organization of Research, held in Israel, January 1970.) Pp. 353. (National Council for Research and Development: Jerusalem; Gordon and Breach: New York and London, 1972.) n.p.

THIS volume is a collection of twenty-two main papers delivered at the Israel–Latin American symposium in 1970, plus an extract from the discussion. It suffers from some of the usual defects of such a volume, notably the absence of an index and the unevenness of the contributions which range from over-familiar generalities about science policy to important specific evidence of Israeli experience.

Israel commands special attention from all interested in science policy. She provides a model for those starting science-based industries from scratch, and is endowed with a promising new government organization, the National Council for Research and Development, set up after Professor Katchalski's[1] enquiry of 1966–68. But Israel has also managed to look outward, and help the developing states with her knowledge and skills, though recent experiences in Uganda and in Chad may have been discouraging.

Yet Professor Sabine's view of Israel in his keynote speech at the symposium as "an important pilot plant for the developing countries of the world" claims too much. Even allowing for Israel's special difficulties, these other countries do not have the human and educational resources, the important oversea connexions and the sheer abilities that make Israel unique. Professor Sabine himself reveals he is only a "recent" Israeli, coming from the US, and elsewhere Professor Keynan says 80 per cent of the Hebrew University's research budget comes from abroad. Professor Bergmann indeed emphasizes that the characteristic difference between Israel and other countries is that she had a scientific establishment long before she had a political establishment. Dr Arnon says firmly that Israel cannot provide recipes for other countries: "every country must solve its own problems".

Many of the contributions deal with universal problems: government scientific organization; government finance for research and development; the role of scientists in government and management; the evaluation of research; the argument for independent scientific research even in small countries; industrial attitudes to research; the relationships between pure and applied research, between government and industry and between universities and industry. On this last item, the influence of the Weizmann Institute and its "industrial park" is especially interesting. The role of defence in science policy is, unfortunately, only touched upon; this is clearly a field where a detailed account of Israel's experience would be illuminating.

The most rewarding chapters, for me, are those in the middle, which deal with agriculture and science-based industry. Dr Arnon's two chapters on agriculture are analytical and practical, lively and illuminating. He cites the development of Arab agriculture in Israel as something really relevant to developing countries, for it shows how knowledge of improved technology *per se* and even the availability of improved inputs has no influence whatsoever on traditional agriculture until a whole set of circumstances change. The industrial case studies are also most interesting, while Mr Tolkovsky's paper on private investment in science-based industries stands high for its wisdom and practical experience.

The volume as a whole reflects the vitality of science and industry in Israel. If too little is said of Latin America, we may take it that the participants from those countries were more anxious to listen than to speak. If so, they would have learned much.

MARGARET GOWING

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[1] Professor Katchalski is now President of Israel.

Seeing Infrared

Laboratory Methods in Infrared Spectroscopy. Edited by R. G. J. Miller and B. C. Stace. Pp. xxi+375. (Heyden: London and New York, September 1972.) $18; £6.25.

THIS is the second edition of a well-known book on the experimental techniques associated with infrared spectroscopy. The infrared method is a very powerful one for structural determination and, in suitable instances, for quantitative analysis. In large measure, its power is to be found in its capability for dealing with an extremely wide range of types of samples—from gases to crystalline solids and from fibres to pastes. In many cases points of experimental technique make the vital difference between success and failure in this type of work and, despite a 25-year period of development, considerable progress continues to be made.

The coverage in topics in this second edition is considerably wider than before and, with the exception of the omission of a specific chapter on infrared emission spectroscopy, is really comprehensive. It includes also chapters on Raman spectroscopy and Fourier transform infrared spectroscopy. The same care as before has been shown in choosing contributors who are highly experienced in the various techniques, and the editors have been skilful in guarding against unnecessary overlap in the different contributions. It is a pity, however, that there is no index.

Without question this is the best as well as the most up-to-date account of infrared experimental techniques for the chemical spectroscopist. It is very highly recommended and deserves a wide sale.

N. SHEPPARD

The Purines

The Purines: Theory and Experiment. Edited by Ernst D. Bergmann and Bernard Pullman. (Proceedings of a symposium held at Jerusalem, April 1971.) Pp. 61. (The Israel Academy of Sciences and Humanities: Jerusalem, November 1972.) (Distributed by Academic: New York and London.) $29.

Regulation of Purine Biosynthesis. By J. Frank Henderson. Pp. xv+303. (American Chemical Society: Washington DC, 1973.) $12.95.

THE first of these books presents a remarkable instant picture of the state of knowledge of purine structure and chemistry at the beginning of the 1970s. By their success in drawing together the majority of outstanding workers in this field, and asking them to present a contemporary contribution to the theoretical and experimental aspects of the purines, the organizers of the symposium have captured, and preserved for society in this one volume, a fascinating collection of forty-eight original papers. There was apparently no division into subject areas during the symposium but a subsequent examination reveals four main areas into which the bulk of the papers could be placed: (1) The molecular structure of simple purines, purine adducts, nucleosides and nucleotides; (2) properties of the purines, for example, tautomerism, site of protonation and metal attachment, photochemical reaction; (3) the organic, biochemical, and medicinal chemistry of purines and related compounds; and (4) papers with a predominantly theoretical content, in particular quantum theory and molecular orbital calculation. The application of physical and physicochemical methods for structure determination is essential to current work and the supreme importance in this field of X-ray crystallography was shown by the fact that more than one quarter of the total papers were largely concerned with this technique. In addition several papers referred to i.r., u.v., n.m.r. and fluorescence spectrometry and electrical conductivity, e.s.r., mass spectrometry, and circular dichroism.

Undoubtedly the first of the above subject areas is the one at present undergoing the most rapid rate of increase in knowledge and the one in which the most exciting papers appeared. To single out any small number of papers must be somewhat invidious, but four of the most fascinating to a reader of the book are "The Structure of Adenosine Triphosphate" by the Cambridge team of O. Kennard, W. W. Isaacs, W. D. S. Motherwell and D. G. Watson, "The Stereochemistry of Actinomycin Binding to DNA" by Henry M. Sobell, "Solid-State Stacking Patterns of Purine Bases" by C. E. Bugg and "Conformations in Polynucleotides" by S. Arnott.

All the papers were submitted to a critical but appreciative audience and it is one of the merits of the book that the discussion is included. Particularly relevant criticisms to many of the papers came from M. Sundralingam and W. Pfleiderer and two recurrent themes discussed on several occasions were the forces controlling base stacking and the positions of protonation in several related purines. The two editors, E. D. Bergmann and B. Pullman, were themselves authors of papers which linked theoretical propositions with practical and they are to be congratulated for their editorial work.

The book is well produced with clear formulae, tables, diagrams and photographs, but the index is somewhat short for a book of this size. A final chapter entitled "Concluding Remarks", by E. D. Bergmann, is useful for a rapid check of the main theme of each paper nd the summary which several authors ive could with advantage have been ade mandatory for all.

Regulation of Purine Biosynthesis is timulating and exciting book which uld be read by all those interested urine chemistry and in the study of ctural problems in heterocyclic nistry in general. *The Chemical nographs* published by the American mical Society since 1921 are in- d to make available to chemists rough treatment of a selected area form usable by persons working re or less unrelated fields to the at may correlate their own work larger area of physical science stimulate further research in the c field treated. Professor Hender- as undoubtedly been thorough in eview of the biochemical literature the production of the monograph *lation of Purine Biosynthesis.* In al chapter, however, he is inevitably ght to the conclusion that practic- othing is known of how regulatory anisms operate in intact cells and the different regulatory mechan- for the discrete steps are integrated. furthermore obvious from reading ook that very little is known of the hanism at the molecular level of n of the eleven steps in the biosynthetic pathway from inosine-5′-phosphate to inosinic acid.

Possibly because the author attempted to refer to so many original papers the book seems at times to be a catalogue of summarized findings from other workers. One would have welcomed a more critical approach and a call for help from theoretical chemistry, X-ray crystallography, ^{13}C n.m.r. spectroscopy and other potentially useful areas. There are several printing errors, particularly in the formulae, and pyrimidinones, pteridinones, purinones and mercaptopurines are all incorrectly represented in their unlikely tautomeric structures.

Regulation of Purine Biosynthesis shows how too narrow an approach by very many workers has not answered important biochemical questions. The Jerusalem symposium demonstrates the types of tool which are effecting major advances in other biochemical areas and which must surely be valuable in the solution of all heterocyclic biochemical problems.

D. G. Wibberley

Neutral Mutations

Theoretical Aspects of Population Genetics. By M. Kimura and T. Ohta. Pp. ix+219. (Princeton University: Princeton, New Jersey, 1971.) $12.50.

This is an excellent book, which should be read by everyone interested in population genetics. The authors are two of the world's outstanding theoretical geneticists, and this book is essentially an account of their contributions to the field over the past five years or so. It is admirably clear and concise; the non-mathematically minded are catered for by the relegation of most of the mathematical proofs to an appendix. In chapter 1, the problem of the fixation of mutant genes is treated; this is used in chapter 2 in discussing the interpretation of observations on the rate of evolution as measured from protein sequence data. Later chapters deal with the concept of effective population size, the theory of genetic load, two-locus problems, the maintenance of variability in populations, and the adaptive significance of sex.

Kimura and Ohta believe that most nucleotide substitutions in evolution are due to the random fixation of selectively neutral alleles, and that protein polymorphisms revealed by electrophoresis merely represent a transient phase of this molecular evolution. Most of the mathematical results described in this book have been developed in order to show that this theory can account for the known facts of protein variation and evolution. Kimura and Ohta do a very good job of presenting this view, and only the most dyed-in-the-wool panselectionist can fail to be impressed. Nevertheless, there are some facts which are hard to fit into a neutral scheme of things, notably Prakash and Lewontin's discovery of non-random associations between inversions and protein variants in *Drosophila pseudoobscura.* It is a pity that these observations are not discussed in this book.

Many criticisms have, of course, been levelled against the authors' arguments for neutral mutations. In this book, most of them are dealt with quite convincingly. There does, however, seem to be an inconsistency which is hard to overcome. Kimura and Ohta show that, on the neutral mutation theory, the rate of amino acid substitution in evolution is equal to the rate of origin by mutation of new alleles affecting protein structure. If the data from proteins which have been sequenced are taken as representative, the average rate of mutation to neutral alleles per locus per generation can be computed. Kimura and Ohta find that this rate is less than one-tenth the order of magnitude of the mutation rates per locus measured experimentally in higher organisms. These mutation rates are based almost exclusively on rates of mutation to deleterious alleles. They therefore conclude that "this suggests that the neutral mutations constitute a rather small fraction of the total mutations".

Now the rate of neutral mutation for the whole genome can be estimated by multiplying the rate per nucleotide site by the total number of sites in the genome, calculated from the DNA content of sperm. For man, Kimura and Ohta estimate that between sixty and seventy-five neutral mutations occur per genome per generation. They argue that this means that "nucleotide substitution has an appreciable effect on fitness in only a small fraction of DNA sites", otherwise "the mutational load must be unbearably high for human populations".

This is a puzzling contradiction, which is difficult to resolve without serious damage to some other parts of the case for neutral mutations. For example, if one assumes that much of the DNA is functionless, the argument that most amino acid substitutions cannot be adaptive (because there would otherwise be too high a substitutional load) loses its force. Perhaps Kimura and Ohta would argue that observed mutation rates are biased in favour of loci which are known to have mutated and which therefore must have higher than average mutation rates. This can be tested by measuring mutation rates for electrophoretically detectable loci. Some data of this sort have been published by Mukai and by Kojima, and these agree quite well with usual mutation rates.

I would emphasize again that this is

an important and well-written book. The question of the possible selective neutrality of most protein variation is one of the most interesting in contemporary biology. Kimura and Ohta have greatly refined the mathematical tools needed for tackling this problem, and have made us all much more critical in our attitude towards evidence in favour of selection. This book is a valuable account of their work.

BRIAN CHARLESWORTH

First Know Your Rocks

Petrology of the Igneous Rocks. By F. H. Hatch, A. K. Wells and M. K. Wells. Rewritten thirteenth edition. Pp. 551. (Thomas Murby: London, April 1973.) £7.40.

MOST students of petrology will be familiar with "Hatch and Wells", which has enjoyed a long and honourable career as a student textbook since its first appearance in 1891. Even those nurtured on the rival "Harker" were (and indeed still are) advised, when sufficiently mature, to consult this work for its excellent account of igneous activity in the British Isles, although they may have been firmly directed to read no further. In this book the emphasis has always been placed firmly on a clear interpretative exposition of petrography based on evidence that can be gleaned from the rocks. Despite the esoteric temptations offered by some modern treatments or by the *art nouveau* of plate tectonics, the Wells family has adhered firmly to its belief that any appreciation of the global setting of petrology can only be achieved properly if the student first knows his rocks.

During the twelve years that have intervened between the twelfth and thirteenth editions, our knowledge of the evolution of the Earth's crust has been completely transformed: no less dramatic have been the advances in the fields of experimental petrology and geochemistry which have enabled a degree of certainty, hitherto lacking, to be given to petrogenetic theory. It is clear that these changes have been appreciated by the authors, although they have not exactly been swept off their feet. Although much of the otiose crystallographic material has been removed, surely it is petrographic mineralogy that is needed; all the structural mineralogy should have gone as well. A chapter on the geological setting of igneous activity has been added and those dealing with basalts, andesites, trachytes and rhyolites have been completely revised and extended. A new approach has been made to the vexed problem of rock classification and nomenclature. Although a satisfactory classification of salic rocks is presented, no clear scheme is apparent for the intermediate and mafic varieties; a comprehensive classification table should have been included. New material has been added to other chapters where needed and references have been updated. All this has been achieved without substantially increasing the size: alas, the same cannot be said of the price.

In view of the extensive rewriting, it is a pity that the opportunity was not taken to increase the page size, for the book does not lie open well. References could have been found more easily and the appearance would have been improved if the numerous footnotes had been replaced by a full bibliography. It is pleasant to note that the fine micro drawings have been retained; these portray so much more clearly than do photomicrographs the textural features of the rocks. The treatment of chemical data is less satisfactory, for although many tables of rock analyses are included and the principles of both the CIPW Norm and Niggli values are explained, they are little used. Variation diagrams are treated very briefly, and although simple phase diagrams are introduced early in the book they are rarely used to explain the petrology.

In spite of these criticisms the authors deserve to be congratulated on presenting a well balanced, interesting and thoughtful modern account of igneous petrology written for the level of a second year student.

I. D. MUIR

Interstellar Scatter

Light Scattering Functions for Small Particles with Applications in Astronomy. By N. C. Wickramasinghe. Pp. 506. (Adam Hilger: London, 1973.) £12.

AFTER a short summary of observational evidence for interstellar dust and the extinction of electromagnetic radiation by that dust, followed by a similarly short account of the computational procedures used to calculate the efficiency factors for scattering, absorption and back-scattering and the forward directivity of scattering of spherical cylindrical and composite dust grains, the book goes on to detail these factors over a wide range of refractive and absorptive indices and radius of particle in wavelength units. Graphs of the results are given for selected cases. Specific computations are given for ice, iron and graphite and for composites with graphite and iron cores.

From this brief description it will be obvious that this is a book for the aficionado, even if these should include students of meteorology and colloid science, as claimed. It is a pity that computations were not given for the currently favoured silicate material. Nevertheless, the book covers a very wide range of conditions and will be useful both for itself and as a spur for others contemplating similar computations.

The book is well and attractively produced, the tables being of tidily laid out computer output. Its main fault is the price, surprisingly high for a book of over 90% of photolitho content. The user will probably borrow his copy from his library, which ought to have one.

H. SEDDON

Hyperfine Structure

Theory of Hyperfine Structure of Free Atoms. By Lloyd Armstrong, Jr. Pp. ix+209. (Wiley: New York and London, March 1972.) £7.

TECHNICAL progress in atomic spectroscopy in the last twenty years, the development of highly monochromatic sources of radiation such as atomic beam frequency standards, masers and lasers, and sophisticated spectrometers capable of detecting transitions between individual atomic states, has allowed a great increase in the precision with which hyperfine interactions in free atoms can be measured. It is not surprising that there has been a corresponding development in the rigour and complexity of the theory to such an extent that it is now difficult for an active research worker to follow developments without reference to the original literature associated with severa
diverse fields. This volume represen
what is probably the first comprehensi
review of the theory of fine and hyp
fine interactions. The interactions
tween the nucleus and the electrons
analysed using a consistent set of t
niques and clearly defined approxi
tions in such a way that the relatio
between the theory of hyperfine i
actions and the theory of atomic
structure is stressed. The basic th
of angular momentum, second qua
tion, relativistic electrons an
groups are presented in the firs
and then used to develop the th
atomic structure and the hyperfin
action. There is one chapter
effects of external electric and m
fields and another concerned with
order effects such as the hyperfine
malies associated with nuclear str
and electric dipole moments. The
chapters deal with the hyperfine
actions in one electron and many
tron atoms. The presentation
sophisticated for the average e
mentalist who will probably find
of the techniques used unfamiliar
difficult, but the volume will be of
help to the research worker an
graduate student who needs to stud
subject in depth.

K. E. SMI

CORRESPONDENCE

Weber's Waves

SIR,—I feel impelled to write about the report on the Oxford–Cambridge–London Relativity Seminar (*Nature*, **243**, 61 ; 1973), as the account contains several inaccuracies and might give someone unfamiliar with the current experiments on gravitational radiation some misleading impressions. The reporter seems to have drawn the conclusion that it has already been shown that Weber's gravitational wave detectors are not detecting gravitational waves when in fact this degree of finality goes rather beyond the implications of currently available experimental data. It is true that Weber's experiments have not been confirmed, and indeed the first results from three or four experimental groups make it appear more probable that Weber's interpretation of his data is mistaken than that it is correct; but I do not think that experimenters working in the field have claimed this was a definite conclusion yet, and it seems ather premature for the *Nature* porter to assume it and incorporate it strongly in the title of his article.

As the report mentions a review which ave at Oxford of the current experintal situation, I think I should point that some of the statements which ht appear to be attributed to myself inaccurate and do not accord with own views. For example, it is rted that all gravitational wave tors are cooled to reduce thermal —when, although cooled detecre being built, few, if any, are in ion. And I do not think that the sion is inescapable that Weber t detected gravitational waves. ments in this field are long and t, and although one may speculate the probable outcome I think it early to make definite conclu- at this stage.

several places in this article, includhe report of the theoretical discus- I feel there are slight inaccuracies , although small in themselves and bly accidental, do give an overall ession which may not help the rch and may indeed unnecessarily some of the persons involved. ough research on gravitational ation is of a type which may, or may uncover new and unpredicted phenomena, I do not think it helps to overdramatize some aspects of the situation when what is required is patient experimental work together with careful interpretation.

Yours faithfully,

R. W. P. DREVER

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The Mind Diseased

SIR,—Dr Thomas S. Szasz (*Nature*, **242**, 305 ; 1973) writes that, strictly speaking, " . . . disease or illness can affect only the body. Hence there can be no such thing as mental illness". Thus he assumes that mental and bodily functions can be clearly separated, and that the mind is something apart from the body. This appears untenable in the light of modern biology ; the mind cannot be divorced from the organism as a whole. Dr Szasz appears further to say—although I wonder whether he really means it—that "mental patients", who suffered from no recognizable bodily illness, were generally and properly considered to be fakers and malingerers, until Charcot, Janet, and Freud led us astray with the concept of mental illness. I believe that Dr Szasz is wrong, both philosophically and historically.

No doubt many patients in the past were considered malingerers. Before the functions of the thyroid were understood, for instance, victims of hyperthyroidism or myxoedema were probably often placed in this category. Our knowledge of the finer details of the working of the brain is still rudimentary ; the psychiatrist, dealing with a patient who is suffering agonizing distress, may not be able to put his finger on any objectively detectable abnormality in the patient's nervous system or hormonal balance. Are we therefore to say that the patient is not really ill, because no physical or chemical test is yet available, to be correlated with the symptoms? Does illness become real when someone discovers such a test, although it was unreal before?

The idea that nobody before Charcot believed in the reality of mental illness is untenable. Consider, for instance, the cry of Macbeth to the doctor who is treating Lady Macbeth: "Canst thou not minister to a mind diseased?" In this case, certainly, the doctor was helpless, but it is obvious that both Shakespeare and his audience believed in the reality of mental illness.

One famous eighteenth century patient, William Pitt, Earl of Chatham, suffered from occasional manic episodes and from several long periods of terrible depression, when he was completely incapacitated for many months at a time During one such period (1767 68) Pitt was nominally Prime Minister ; he begged George III to let him resign, because he was ill, and the King finally and reluctantly granted his request. Pitt's physician, Anthony Addington, certainly considered the depression as an illness ; his major therapeutic approach was to encourage the development of attacks of gout, a disease to which Pitt was also subject. This was perhaps the eighteenth century equivalent of shock therapy. In any case, both Pitt and his doctor regarded these depressions as real illnesses[1].

Dr Szasz proposes that all those who commit crimes should be dealt with by criminal law. Perhaps ; but there is a difference between the man who kills another to get his money, and one who kills because he believes that his victim was trying to destroy him by evil magic. Perhaps both should be tried by the same judicial procedure, but surely the sentencing of the criminal should take the particular circumstances into account.

I share the concern of Dr Szasz over the outrages inflicted on many patients confined in mental hospitals against their will. A campaign against such cruelty and injustice is entirely in order, but I believe that it will have a better chance of success if it is not entangled with a highly dubious philosophical outlook.

Yours faithfully,

JOHN T. EDSALL

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16 Divinity Avenue,
Cambridge, Massachusetts 02138

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[1] Tunstall, Brian, *William Pitt, Earl of Chatham* (Hodder and Stoughton, London, 1938).

Eddington

Sir,—The letter you published by H. W. Grayson (*Nature*, **242**, 317 ; 1973) must, on internal evidence, be judged to be fraudulent: the strong implication of a pre-1926 date in the letter does not agree with a reference to Sir Arthur Eddington—Professor A. S. Eddington was awarded a knighthood in 1930.

Yours faithfully,

H. Mykura

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University of Warwick,
Coventry, Warwickshire CV4 7AL

Complementarity

Sir,—The book review by E. H. Hutten, "Microphysical Phenomena" (*Nature*, **241**, 220 ; 1973), suggesting the need for a new approach to such problems after fifty years of discussion, has just come to my notice.

Complementarity is invoked, but it is the principle of interdependent incompatibility which needs to be applied. According to this principle, the co-existence of mutually exclusive states is possible when each state determines the other and is thereby instantaneously excluded (like the concepts "position" and "displacement from that position", for instance), so that the two states alternate so rapidly within any given period of time that they appear to co-exist at the same time.

The Greeks worried about this problem, but notoriously never made experiments. Now, some 2,000 years of test results later, it pays to look once more at the problems they posed, in the light of these results.

I have postulated the principle of interdependent incompatibility not as an abstruse hypothesis, but because it is simple, obvious and fits the observed facts better than any other—as I could show, given the chance, or as could be worked out by others who put their mind to it. So far, I have hawked the principle variously, in vain. What good is the demand for a new approach if, when such approach is made, no one wants to know?

Yours faithfully,

U. Light

65 Upper Queens Road,
Ashford, Kent

Fossils of Creation

Sir,—I feel that I really must argue with E. C. Lucas (*Nature*, **242**, 355 ; 1973) on his suggestion that fossil evidence supports the Hebrew creation myth. The old ideas of the "ages" must be dropped ; the age of fishes, the age of the coal forests and so on, have no reality. Fishes appear in the Devonian but must have had a long evolutionary history which has not been preserved. Reptiles and mammals appear in the Perino-Trias, again fully evolved. One cannot interpret the six days of creation in terms of artificial geological epochs; it is taking credulity too far and is interpreting "Neolithic science" in terms of modern science. Why six days? The Neolithic farmer would have known that the phases of the Moon repeated every 28 days. His natural submultiples of numbers would be obtained by halving. The smallest whole number which would be obtained by repeated halving is seven. Seven days becomes a week. Four weeks becomes a month (lunation), thirteen lunations becomes one year of 364 days. Thus he had seven days to the week, four weeks to the month and thirteen months to the year. The "science" of numerology led to giving magical significance to these numbers. I need elaborate this argument no further.

The Neolithic farmer would work for six days and worship on the seventh because the seventh day was "magic". Naturally he would extend such reasoning to the creator who would labour for six days on his creation. As to the order of creation he would start at the "bottom" and work his way up to the "top". The ocean, the land, the heavens. Man, naturally, would occupy a special phase as the last and most perfect of all the creation.

The *Bible* is a collection of stories, songs and legends not necessarily in the correct chronological order. The *Bible*, like the *Iliad*, or Geoffrey of Monmouth's history of the Kings of Britain, contains a strong element of historical fact (or should it be inference?) which we must be careful not to interpret too literally.

I am sorry, but the Hebrew creation myth has no support at all from the fossil record. If Mr Lucas wishes to believe in it then he must evoke an act of faith—a highly unscientific mental process.

Yours faithfully,

J. Saxon

7 Rockwell Terrace,
Thurso,
Caithness

Games with GNP

Sir,—Although grateful to *Nature* for printing my paper "Features of a Closed System Economy" (*Nature*, **242**, 561 ; 1973), I should point out in connexion with the editorial, "Fun and Games with GNP", that Boulding's writing, and especially his books, *Economics as a Science* and *Beyond Economics*, in fact led to my paper, and in both the professor is apparently guilty of the "curiously subjective" error which I am accused of, because he roundly equates GNP to gross national cost with, I think, rather brilliant reasoning based on thermodynamics. My question of gross product to the sum of gross consumption (and the rest used to improve society) is a precise quotation from the professor's book *Economics as a Science* (page 45).

I have gone further than did the professor in analysing "good" and "bad" costs but the idea is his. There is also no difficulty on real cost. The real cost of an activity is the amount of non-renewable resource converted by it from potential energy in the ground to useless and irrecoverable waste (plus the personal effort involved in it). It is obviously akin to entropy in physics although essentially a vector rather than a scalar quantity. The difficulty of avoiding some arbitrariness in drawing the line between activities which obviously increase entropy and those which decrease it (Boulding's negative entropy of "structures of increasing improbability") is because we are dealing with a social science and not physics. But difficulties of definition do not destroy realities.

The illustration given about education is mistaken, and does not invalidate Boulding's argument (or mine). Clearly the contribution of policies towards discouraging the kinds of investment which merely add to further wasteful or unnecessary consumption must in the end be to limit the supply of unnecessary goods—and the consuming public (including the school teachers) can only buy what is available. The difference is clear enough, I think, between the likely behaviour pattern of a potentially rational society and the situation of the artificially stimulated captive consumers so well described in Vance Packard's book, *The Waste Makers*.

The technological optimism of the last paragraph is, I believe, of the wrong kind, and here I draw attention to recent published work of the US Geological Survey relating to energy resources for power production. This makes it clear, I think, that one of the risks of absolute or near shortages could be increasing rigidity, as substitution technology is invariably power hungry. From this kind of thinking I would guess that the oceanic uranium referred to will probably remain where it is! I believe the true direction of future technology is towards more advanced conservative systems as suggested in the last part of my paper, to which no space or regard is given; this, along with quite far-reaching changes in values and habits of mind, which are at present almost hopelessly one-tracked. And are we so sure that we are on the right track, in looking at the possibilities of tearing the Earth apart merely to sustain a high level of activity and a rapidly ageing body of dogma which many economists seem to believe in with almost incredible persistence?

Finally, on relevance to the real world. First, the exercise has to do with the need for major regional projects of the Russell-Spoffard kind on the European side to at least provide the infrastructure of more advanced systems should the cultural difficulties ever be overcome; second, for greater investment in recycling and recovery projects of the kind currently undertaken and publicized by the National Research Development Corporation; and third, it clearly concerns changes in design thinking by the engineering industries, in the direction of durability and reducing obsolescence.

Yours faithfully,
R. E. Overbury

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St Johns Wood,
London NW8

Physical Units

Sir,—An article under the title, "Natural Units in Atomic and Molecular Physics", from Professor R. McWeeny which appeared in your issue of May 25 (**243**, 196; 1973) draws attention to an area in which there is need of common international usage. It has already been announced that the IUPAC Commission on Physicochemical Symbols, Terminology and Units might consider this question and opinions and suggestions have been requested by its Chairman, Dr M. A. Paul, NRC Division of Chemistry and Chemical Technology, National Academy of Sciences, Washington DC 20418, USA. The need for an authoritative examination and agreed recommendation is brought out by McWeeny's article since most scientists feel that if letters such as H, as, aV and aT have internationally agreed meanings as the Henry, attosecond, attovolt and attotesla, it is objectionable for them to have special meanings of hartree, atomic second, atomic volt and atomic tesla in special contexts. What is one's reaction to the statement 1 as=24.1889 as?

Yours faithfully,
D. H. Whiffen

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Announcements

International Meetings

July 1–7, **The Study of Time.** (Secretary, J. T. Fraser, International Society for the Study of Time, P.O. Box 164, Pleasantville, N.Y. 10570, USA.)

July 1–7, **Ninth International Congress of Biochemistry.** (The Secretariat, 9th International Congress of Biochemistry, c/o Svenska Kemistsamfundet, Wenner-Gren Centre, 6tr S-113 46 Stockholm, Sweden.)

July 2–3, **Neutron Activation Analysis Symposium.** (Mr Peter Duggan, Symposium Secretary, Neutron Division, Marconi-Elliott Avionic Systems Limited, Elstree Way, Borehamwood, Herts.)

July 2–6, **The Sun in the Service of Mankind.** (Congrès International The Sun in the Service of Mankind, 28, Rue de la Source, Paris-XVIe, France.)

July 2–6, **Second AIC Congress "Colour 73".** (Professor W. D. Wright, Applied Optics Section, Imperial College, London SW7 2BZ.)

July 3–5, **Conference on Scanning Electron Microscopy Systems and Applications.** (The Institute of Physics, 47, Belgrave Square, London SW1X 8QX.)

July 3–6, **Molecular Motions in Liquids.** (Dr C. Troyannowsky, Secrétaire Général Société de Chimie Physique, 10, Rue Vauquelin, 75, Paris.)

July 4–5, **Anglo-French Symposium—Freeze Etching.** (The Administrator, Royal Microscopical Society, Clarendon House, Cornmarket Street, Oxford.)

July 4–6, **Fourth Canadian Wood Chemistry Symposium.** (Professor R. H. Marchessault, Department of Chemistry, Université de Montréal, C.P. 6128, Montreal 101, Quebec.)

July 5–6, **A Symposium on Genetic and Structural Aspects of Virus Genomes.** (The Meeting Assistant, Society for General Microbiology, Harvest House, 62, London Road, Reading RG1 5AS, Berks.)

July 6, **Symposium—X-ray Microanalysis of Biological Material in the Electron Microscope.** (The Administrator, Royal Microscopical Society, Clarendon House, Cornmarket Street, Oxford.)

July 8–14, **Twenty Years of Health Education: Evaluation and Forecast for the Years Ahead.** (Secretariat, VIIIth International Conference on Health Education, 20, Rue Greuze, 75 Paris 16e, France.)

July 9, **The Structure of Biological Molecules.** (Dr Lars-Johan Norrby, Structure of Biological Molecules, Department of Inorganic and Physical Chemistry, University of Stockholm, Roslagsvaegen 128, S-104 05 Stockholm, Sweden.)

July 9–20, **The Physics of Quantum Electronics.** (Professor Stephen F. Jaco and Marlan O'Scully, Optical Sciences Center, The University of Arizona, Tucson, Arizona.)

July 10–12, **Conference on Video and Data Recording.** (The Information Officer, 8–9 Bedford Square, London WC1B 3RG.)

July 10–13, **Synthesis in Organic Chemistry.** (Dr John F. Gibson, The Chemistry Society, Burlington House, London W1V 0BN.)

July 10–13, **The Fourth International Colloquium on Gasdynamics of Explosions and Reactive Systems.** (International Academy of Astrautics of the International Astrautical Federation, 250 Rue Saint-Jacques, 75 Paris, France.)

July 11, **Biochemistry and Treatment of Depression.** (Dr J. F. Cavalla, Society for Drug Research, John Wyeth & Brother Ltd., Huntercombe Lane South, Taplow, Maidenhead, Berks.)

July 11–13, **The Hundred and Seventy-second Meeting of the Genetical Society.** (Professor D. A. Hopwood, John Innes Institute, Colney Lane, Norwich.)

July 11–13, **European Nutrition Conference.** (Dr E. M. Widdowson, Dunn Nutritional Laboratory, Milton Road, Cambridge CB4 1XJ.)

July 11–13, **British Society for the History of Science—Summer Meeting.** (BSHS Office, 47 Belgrave Square, London SW1X 8QX.)

July 12, **The Annual General Meeting of the British Society.** (BSHS Office, 47 Belgrave Square, London SW1X 8QX.)

July 15–20, **Second International Conference on Culture Collections.** (Dr Antonio Fernando Pestana de Castro, Palacio das Convenções, Parque Anhembi 02012, São Paulo, SP, Brazil.)

July 15–20, **Symposium: Biotransformations and Fermentations.** (John M. Cassady, Ph.D., Chairman, ASP Publicity Committee, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana.)

July 16–19, **Ninth International Shock Tube Symposium.** (Symposium Secretary, Department of Aeronautics and Astronautics, Stanford University, Stanford, California.)

July 16–27, **Capri, Italy.** (Dr E. R. Pike, Royal Radar Establishment, Malvern, Worcs, U.K.)

July 16–28, **The Second International Conference on Permafrost in Yakutsk, Siberia.** (Dr Troy Péwé, Chairman, U.S. Planning Committee, National Research Council, 2101 Construction Avenue, N.W., Washington, D.C.)

July 17–20, **Conference on Pollution Criteria for Estuaries.** (Dr P. R. Helliwell, Department of Civil Engineering, The University, Southampton, SO9 5NH.)

July 17–20, **Biochemistry of Inositol Lipids Control of Fatty Acid Synthesis and Related Metabolism.** (Dr D. N. Brindley, Department of Biochemistry, University of Nottingham Medical School, Nottingham NG7 2RD.)

July 17–20, **Association for the Study of Animal Behaviour.** (Dr A. W. Ewing, Department of Zoology, West Mains Road, Edinburgh EH9 3JT.)

July 22–27, **Symposium on Man-Machine Communication for Scientific Data Handling.** (Secretary of the Task Group, c/o Mr M. K. Ward, International Conferences Office, National Research Council of Canada, Ottawa K1A OR6, Canada.)

July 23–28, **The Fourth International Conference on the Global Impacts of Applied Microbiology.** (Dr Joao S. Furtado, Secretary-General GI AM IV, R. Gabriel dos Santos, 443, São Paulo, SP, Brazil.)

July 24–August 8, **1973 ESRO Summer School on European Manned Space Missions.** (European Space Research Organization, 114 Avenue Charles de Gaulle, 92-Neuilly-sur-Seine.)

July 31, **Protein Synthesis and Muscle Growth.** (Mr Claude Cruse, Business Manager, American Society of Animal Science, 425 Illinois Building, 113 North Neil Street, Champaign, Illinois.)

Reports and Publications

not included in the Monthly Books Supplement

Great Britain and Ireland

Biochemical Systematics, Vol. 1, No. 1, January 1973. Published quarterly. Pp. 1–78. Subscription rates: for libraries, university departments, government laboratories, industrial and other multiple reader institutions £12; $30. Private individuals whose departmental libraries subscribe may obtain the journal for their personal use at a reduced rate of £5; $12. (Oxford and New York: Pergamon Press, 1973.) [123

London School of Hygiene and Tropical Medicine. Report on the work of the School, 1971/1972. Pp. 134. (London: London School of Hygiene and Tropical Medicine, 1973.) [123

Potato Marketing Board. Sutton Bridge Experimental Station Annual Review—1972. Pp. 47. (London: Potato Marketing Board, 50 Hans Crescent, 1973.) [123

The Birds from Your Window. Pp. 31. (Sandy, Bedfordshire: The Royal Society for the Protection of Birds, The Lodge, 1973.) *Gratis.* [123

Mental Health Research Fund. Annual Report and Accounts 1972. Pp. 30. (London: Mental Health Research Fund, 1973.) [123

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National Radiological Protection Board. NRPB-R4: Dose Rates and Depth-Dose Distributions for Beta Particles Emitted by Commercially Available ^{90}Sr/^{90}Y, ^{204}Tl, ^{147}Pm and ^{63}Ni Sources. By T. M. Francis and R. Seymour. Pp. 9. (Harwell, Didcot: National Radiological Protection Board, 1972.) [143

Department of the Environment. Children at Play. (Design Bulletin 27.) Pp. 110. (London: HMSO, 1973.) 80p net. [143

The Council of Engineering Institutions. Annual Report for 1972. Pp. 43. (London: The Council of Engineering Institutions, 2 Little Smith Street, 1973.) [143

Countryside Commission. Landscape Agreements. Pp. 15. (London: Countryside Commission, 1973.) [153

University of London. University College Annual Report, 1971/1972. Pp. 148. (London: University College London, 1973.) [163

Other Countries

Australian Journal of Zoology, Supplementary Series, No. 19: The Primary Types of the Australian Eumastacidae (Orthoptera: Eumastacoidea). By K. H. L. Key. Pp. 40. (East Melbourne: Editorial and Publications Section, CSIRO, 372 Albert Street, 1973.) [123

Report of the Council for Tobacco Research—U.S.A., Inc., for 1972. Pp. 103. (New York: The Council for Tobacco Research—U.S.A., Inc., 110 East 59th Street, 1973.) [123

Australia: Commonwealth Scientific and Industrial Research Organization. The Division of Soils—Biennial Report on Progress, 1970/1971. Pp. 77. (East Melbourne: CSIRO, 1973.) [123

Records of the Australian Museum. Vol. 28, No. 11: Earthworms (Megascolecidae: Oligochaeta) from Mount Kosculsko, Australia. By B. G. M. Jamieson. Pp. 215–252. Vol. 28, No. 12: *Typton australis* SP. NOV., a New Pontoniinid Shrimp from the Great Barrier Reef, Australia. By A. J. Bruce. Pp. 253–263. Vol. 28, No. 13: A New Species of *Notuchus* (Homoptera: Fulgoroidea, Delphacidae) from Lord Howe Island. By R. G. Fennah. Pp. 265–267. Vol. 28, No. 14: Galatheidea (Crustacea, Decapoda, Anomira) Collected by the F.I.S. *Endeavour*. By Janet Haig. Pp. 269–289. (Sydney: The Australian Museum, 1973.) [123

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